Project title:	Tomato: application of next generation diagnostics for improved detection and understanding of root diseases
Project number:	PC 281a (Phase 1)
Project leader:	Dr Tim O'Neill, ADAS
Report	Final Report, December 2013
Previous reports:	None
Key staff:	Sean Allison, Chloe Whiteside and Sarah Mayne, ADAS George Scott, University of Nottingham Professor Matt Dickinson, University of Nottingham
Location of project:	Commercial nurseries, Norfolk, Sussex, Yorkshire University of Nottingham
Industry representatives:	Dr Phil Morley, TGA Mr Paul Simmonds and Mr Robert Farthing, Cornerways Nursery, Norfolk
Date project commenced:	1 October 2012
Expected completion:	31 December 2013

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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CONTENTS

GROWER SUMMARY

Headlines

- Several fungal and oomycete pathogens were commonly and consistently detected in the rhizosphere of rockwool grown crops, yet the grafted plants grew well with no obvious symptoms of root or vascular disease.
- LAMP diagnostic assays were developed for on-site rapid detection of *Botrytis cinerea*, *Colletotrichum coccodes* and *Fusarium oxysporum* in this project.

Background and expected deliverables

Root diseases pose a serious threat to tomato production with increased risk where irrigation run-off is recycled. Fungicides previously used for root disease control are no longer approved or very restricted. Growers generally wish to control root diseases without the use of fungicides. 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 microorganisms. Project PC 281 using the molecular method T-RFLP revealed a tremendous diversity of microorganisms on tomato roots and variations between crops. Building on information gained in PC 281, this project aimed to apply next generation diagnostics to improve detection, understanding and control of tomato root diseases. Earlier diagnosis will permit earlier intervention. The aims of the project were: (1) to develop a laboratory based microarray for use in monitoring around 50 taxa of root microorganisms over a full season on rockwool-grown tomato and in irrigation water; (2) to utilise a portable on-site rapid diagnostic kit that is able to detect 12 microorganisms simultaneously with a high degree of specificity to supplement microarray tests to add higher specificity where required.

The expected deliverables from the project were:

1. Knowledge of when pathogenic fungi infect tomato roots and for how long they are present before symptoms occur on roots or in the crop (and whether they can occur without the occurrence of symptoms).
2. Knowledge of the combinations of pathogenic and non-pathogenic fungi and bacteria occurring on tomato roots in rockwool crops and how they change over a season.

3. A comparison of microorganism populations on tomato roots in crops grown with run-to-waste and recycled irrigation solution.
4. Knowledge of how well the microarray (used alone or with the Lamp-based diagnostic) can identify the cause of root rots and stem base vascular infections in tomato

Summary of the project and main conclusions

Objective 1 – Validate microarray for tomato root microorganisms

In a separate project (Nottingham project reference 101139) funded jointly by the University of Nottingham and Fera, a microarray has been developed for the detection of 53 fungal and oomycete species, 7 genera of fungi and oomycetes, 8 species and 6 genera of bacteria and two species and a genus of root-knot nematodes. All the microorganisms represented on the array are known to occur on tomato roots. Within this HDC project the DNA from 105 microorganisms representing 75 species and 36 genera were used to validate the array.

The primer sequences used in the array were each designed for a particular microbial species or genus known to occur on tomato roots. It is possible that the same sequence as used for one of the microarray targets may occur in a different microorganism resulting in a cross-reaction (i.e. a false positive result). The greatest level of confidence can be given in the microarray tests to those primers which were self-validated and did not show a very strong cross-reaction (Table 1).

Table 1. Microarray target organisms which were self-validated and showed little or no cross-reaction

Pathogens	Saprophytes	Bacteria
Alternaria solani	***Alternaria* spp.**	*Agrobacterium rhizogenes*
Aspergillus flavus		
Aspergillus terreus		
Colletotrichum coccodes	*Cadophora* spp.	
	Chaetomium cochliodes	*Bacillus amyloliquefaciens*
Fusarium solani	*Fusarium oxysporum*	*Bacillus subtilis*
Plectosphaerella cucumerina	***Exophiala pisciphila***	
Rhizoctonia solani	*Gliocladium roseum*	*Erwinia* sp.
Thielaviopsis basicola	***Penicillium chrysogenum***	
Verticillium dahliae	***Phoma* spp.**	***Ralstonia solanacearum***
	Trichoderma harzianum	*Rhizobium* sp.
	***Trichoderma* spp.**	

Names in bold showed no cross-reaction.

Objective 2a – Monitor rhizosphere microorganisms over a season

Occurrence of microorganisms

Rhizosphere microorganisms were monitored using a microarray every 2-3 weeks from January to October 2013 in three tomato crops grown on Maxifort rootstock on rockwool slabs. Nursery A in West Sussex grew on a run-to-waste (RTW) irrigation system; nursery B in Norfolk on a closed irrigation system with part of the drainage water recycled through a slow sand filter (pSSF); nursery C in Yorkshire on a closed irrigation system with all drainage water recycled through a Slow Sand Filter (fSSF). The number of microorganism taxa detected on roots on one or more occasion was 26, 42 and 37 at nurseries A, B and C respectively. Four potential pathogens (*Fusarium oxysporum, Fusarium redolens, Plectosphaerella cucumerina, Verticillium nigrescens*) and four saprophytic taxa (*Aspergillus flavus, Olpidium brassicae, Penicillium* spp., *Petriella asymmetrica*) were detected at relatively high abundance in all crops and on most samples. *Colletotrichum coccodes* (cause of black dot) and *Rhizoctonia solani* were commonly detected only at nursery B; *Verticillium albo-atrum* was commonly detected only at nursery C. Eight *Pythium* spp. were detected with four of them (*P. diclinum, P. irregulare, P. myriotylum, P. paroencandrum*) common in all crops. The detection of some of the potential pathogens on

roots in rockwool crops was unexpected: *Colletotrichum acutatum, Rhizoctonia solani, Pyrenochaeta lycopersici*. Examination of the data across all crops showed the fungal and bacterial species diversity on roots declined as the season progressed whereas that of oomycetes increased. There was no evidence of leaf yellowing, plant wilting or plant death due to root disease despite the occurrence of several potential pathogens on roots for many weeks. These results suggest that tomato crops grown on Maxifort rootstock on rockwool slabs can tolerate a certain level of one or more root pathogens for a considerable time without evident adverse effect on crop growth. Possibly the inoculum level of pathogens was insufficient to cause serious root damage; and/or rootstocks were able to rapidly produce new roots to compensate for diseased roots.

Comparison of irrigation systems

The three irrigation systems represented by the three nurseries in this study appeared to have an effect on rhizosphere microorganisms present at those sites. However, it is important to note that some of the differences may be explained by site differences other than irrigation system. Nursery A irrigation water was run-to-waste (RTW) and roots from this crop had significantly less species diversity across the year compared to the other two systems. A significant difference in the mean species diversities was also found between the pSSF and fSSF systems for both the combined (all microorganisms) and fungal diversities from the root samples. Roots sampled from Nurseries B (pSSF) and C (fSSF) generally held a greater number of taxa, with the full SSF system having fewer rhizosphere microorganisms. Rhizosphere microorganisms were monitored every 2 months at two additional nurseries, where the recycled water was treated by UV (D) or heat (E). These two nurseries were both growing on the rootstock Emperador, rather than Maxifort. Differences in fungi and oomycetes were more pronounced in the treated solution sampled than in the rhizosphere itself, suggesting that though treating irrigation water removes a number of pathogenic species, its recycling through the crop allows them to be picked up again. There were no significant differences in levels of bacteria detected between the two treatment systems.

Objective 2b – Investigate tomato root diseases by microarray and conventional tests

Work on this aspect was limited by the lack of root disease problems in rockwool crops in 2013, probably due in part to the switch by most growers to growing on rootstocks. Use of the microarray detected more potential pathogens than conventional tests. There was little

difference in the number and level of potential pathogens as determined by microarray between 'poor' plants and 'good' plants in the same area. Root disease problems were generally associated with a complex of pathogens; diseased roots generally had a lower microbial diversity than 'good' roots.

Objective 3 – Examine microorganism population changes with reference to root zone physical environment

The root zone environment physical factors (pH, EC, temperature) measured by the three host nurseries remained within acceptable range throughout crop production. The rhizosphere microbial populations at each site were relatively stable throughout crop production with respect to species diversity. There was no obvious crop production or rhizosphere physical factor to hypothesise as a cause of a reduced species diversity at all three sites in February; possibly the fall in species diversity at this time was an artefact introduced during laboratory tests. Specific experiments designed to alter one physical factor, for a defined period, offer better prospects for investigating the effect of root zone physical factors on rhizosphere microbial populations.

Objective 4 – Develop and validate LAMP assays to discriminate closely related taxa

Potential targets for in-house point-of-care diagnosis were first identified during meetings with tomato growers. Real-time LAMP assays, using the Genie II (Optigene UK) portable Lamp machine were then developed and validated for *Botrytis cinerea* (grey mould), *Colletotrichum coccodes* and *Fusarium oxysporum*. The assays were then validated using DNA from closely related microorganisms and those found to be common on tomato roots. The tests gave positive results on tomato leaf and root samples naturally infected with these fungi. The assays therefore represent point-of-care tests for these pathogens that can be used to provide positive identifications within 30 minutes of testing.

Financial Benefits

Root diseases of tomato are numerous, widespread and potentially devastating. Plant losses due to root disease have cost over £50,000 on 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. At least 66 fungal pathogens and four bacterial pathogens have been found associated with roots or growing media of tomato plants around the world. In the UK, the most common root diseases are Pythium root rot, Phytophthora root and stem base rot, corky root

rot, black dot root rot, Fusarium wilt, Fusarium crown and root rot and Verticillium wilt; a range of other diseases occur from time to time. 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.

Non-pathogenic fungi and bacteria also occur in the root environment and can influence occurrence of root diseases. Obtaining good information on the occurrence and levels of rhizosphere microorganisms, either pathogens or saprophytes, has, until the recent advent of molecular methods, been difficult, time-consuming and relatively expensive.

Action points for growers

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. The use of grafted tomato plants appears to reduce greatly the risk of severe root disease. We consistently detected several plant pathogens in the roots of Maxifort and Emperador and yet the crops grew and yielded well, with no yellowing, wilting or stunted growth.
2. Tomato plants can have a diverse microbial population on roots, including potential pathogens, even before they are planted onto slabs.
3. Rhizosphere microbial diversity was greater on plants grown with recirculation of the nutrient solution than with the solution run-to-waste. It is an ecological principle that a diverse community is likely to be more resistant to change than a simple community.
4. Although many microorganisms species are present on tomato roots at planting and persist throughout cropping, additional species, including pathogens, (e.g. *Colletotrichum coccodes*; *Verticillium albo-atrum*) may occur during crop production. This indicates a potential benefit from maintaining disease precautions during crop production.
5. *Colletotrichum acutatum*, which is usually reported as a fruit pathogen, was commonly detected on roots. Identification was confirmed by barcoding. Possibly this fungus can also infect and damage roots.
6. *Plectosphaeralla cucumerina* (*Fusarium tabacinum*) was 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, there

is a recent report from South Korea of the fungus causing wilting and death of tomato. Growers should be alert to any reports of *Plectosphaerella* associated with a tomato disease in Europe.

7. Nurseries can differ in the range of pathogens commonly found on tomato roots. There is evidence elsewhere that some pathogens (e.g. *Verticillium albo-atrum*) can persist on a nursery after an outbreak of Verticillium wilt.

8. The detection of several potential pathogens on roots from when plants are planted on slabs, the continued occurrence of these microorganisms through cropping, and the lack of any visible deleterious effect on crop growth at any of the monitored sites, raise the question of how important is hygiene with regard to control of root-infecting pathogens when grafted plants are grown on rockwool slabs? For example, are there potential savings from a reduction in some aspects of hygiene measures between crops, and from treating re-cycled water during cropping? However, 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.

9. 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.

10. 'LAMP' diagnostic assays have been developed in this project that permit very accurate, sensitive and rapid on-site tests for three tomato pathogens: *Botrytis cinerea*, *Colletotrichum coccodes*, *Fusarium oxysporum*. Assays have been developed elsewhere for *Pepino mosaic virus* (3 strains) and *Pythium aphanidermatum*. Growers should be alert for laboratories (e.g. Fera) or commercial companies (e.g. Optigene) offering these as chargeable tests.

SCIENCE SECTION

Objective 1 – Validate microarray

Introduction

Previous work carried out in the project PC 281 gave insight into the microbial populations associated with the commercially cultivated tomato rhizosphere. This information was used to develop a small scale microarray focused primarily on fungal and oomycete pathogens but also including a selection of saprophytic fungi and pathogenic/non-pathogenic bacteria. The array, largely developed in a separate project funded by the University of Nottingham and FERA, was used to give increased frequency, resolution and a semi-quantitative insight into the microbial population of the rockwool tomato rhizosphere.

Materials and Methods

Development

The microarray was developed focusing on the internal transcribed spacer (ITS) and the intergenic spacer (IGS) of the eukaryotic and prokaryotic rDNA, respectively. These regions are analogous between the eukaryotes and prokaryotes and differ only slightly in their architecture. The ITS and IGS regions were chosen due to their conservation within species and ability to discriminate between species.

Computer analyses utilising genetic database GenBank and bioinformatics software MEGA 5.1 were used to identify regions of heterogeneity from which to target specific organisms. These sequences along with an array plan were sent to Alere$_{TM}$, Germany, who manufactured the array.

Table 1. Probes and their targets on the microarray

Probe	Target	Probe	Target
AGRH	*Agrobacterium rhizogenes*	PEBR	*Penicillium brevicompactum*
AGTU	*Agrobacterium tumefaciens*	PECH	*Penicillium chrysogenum*
ALSO	*Alternaria solani*	PEGR	*Penicillium griseofulvum*
ALTE	*Alternaria* spp.	PEUN	*Penicillium* spp.
ARME	*Armillaria mellea*	PEVA	*Penicillium variabile*
ASFL	*Aspergillus flavus*	PHAR	*Phytophthora arecae*

Table 1. Cont'd

Probe	Target	Probe	Target
ASSY	*Aspergillus sydowii*	PHCI	*Phytophthora cinnamomi*
ASTE	*Aspergillus terreus*	PHCR	*Phytophthora cryptogea*
ASUS	*Aspergillus ustus*	PHNI	*Phytophthora nicotianae*
BAAM	*Bacillus amyloliquefaciens*	PHOM	*Phoma* spp.
BACT	*Bacteria universal*	PLCU	*Plectosphaerella cucumerina*
BASU	*Bacillus subtilis*	PLCU2	*P. cucumerina*
CASP	*Cadophora* spp.	PRGO	*Pratylenchus goodeyi*
CESP	*Cephalosporium* spp.	PRUN	*Pratylenchus* spp.
CHCO	*Chaetomium cochliodes*	PSSY	*Pseudomonas syringae*
CHSP	*Chaetomium spp.*	PSUN	*Pseudomonas universal*
CLMI	*Clavibacter michiganensis*	PYAP	*Pythium aphanidermatum*
CLSP	*Cladosporium* spp.	PYAR	*Pythium arrhenomanes*
COAT	*Colletotrichum acutatum*	PYDE	*Pythium debaryanum*
COCO	*Colletotrichum coccodes*	PYDI	*Pythium diclinum*
COCO2	*C. coccodes*	PYEC	*Pythium echinulatum*
DILY	*Didymella lycopersici*	PYIR	*Pythium irregular*
DOMI	*Doratomyces microsporus*	PYLY	*Pyrenochaeta lycopersici*
ECOL	*Escherichia coli*	PYLY2	*P. lycopersici*
ERSP	*Erwinia* spp.	PYME	*Pythium megalacanthum*
EXPI	*Exophiala pisciphila*	PYMY	*Pythium myriotylum*
EXXE	*Exophiala xenobiotica*	PYOL	*Pythium oligandrum*
FUNG	*Fungal universal*	PYPA	*Pythium paroecandrum*
FUOX	*Fusarium oxysporum*	PYTO	*Pythium torulosum*
FUOX2	*F. oxysporum*	RASO	*Ralstonia solanacearum*
FURE	*Fusarium redolens*	RHIZ	*Rhizobium* spp.
FURE2	*F. redolens*	RHOR	*Rhizopus oryzae*
FUSO	*Fusarium solani*	RHSO	*Rhizoctonia solani*
FUSO2	*F. solani*	SPSU	*Spongospora subterranea*
GIRO	*Gigaspora rosea*	THBA	*Thielaviopsis basicola*
GLIN	*Glomus intraradices*	THBA2	*T. basicola*
GLRO	*Gliocladium roseum*	TRHA	*Trichoderma harzianum*

Table 1. Cont'd

Probe	Target	Probe	Target
MEIN	*Meloidogyne incognita*	TRUN	*Trichoderma spp.*
MYRO	*Myrothecium roridum*	TRVI	*Trichoderma viride*
NEUN	*Nematode universal*	VEAL	*Verticillium albo-atrum*
NISP	*Nitrospira* spp.	VEDA	*Verticillium dahliae*
OLBR	*Olpidium brassicae*	VENI	*Verticillium nigrescens*
OOMY	*Oomycete universal*	XANT	*Xanthomonas* spp.
PALI	*Paecilomyces lilacinus*	YESP	*Yersinia* spp.
PEAS	*Petriella asymmetrica*		

Validation

The array was validated by running DNA extractions from cultures, identified via both classic and molecular methods, on the array. The organisms used were both specific to the array, closely related and distantly related species to allow relative levels of specificity for each probe to be inferred. Cultures were provided by The University of Nottingham, FERA and ADAS.

Table 2. Microorganisms used in array validation and their genetic similarity to the ITS/IGS sequences of reference microorganisms in NCIB's GenBank

ID	Microorganism	Homology (%)	Accession
101	*Fusarium oxysporum*	100	JF807394
102	*Verticillium dahliae* var. *longisporum*	99	GQ495792
103	*Ilyonectria radicicola*	99	HQ840390
105	*Plectosphaerella cucumerina*	99	HQ248206
106	*Fusarium oxysporum*	100	JN020659
108	*Gliocladium roseum*	100	KF313107
109	*Mortierella gamsii*	99	GU934542
110	*Penicillium subrubescens*	100	KC346344
112	*Botrytis cinerea*	100	GQ149477
113	*Mortierella elongata*	99	FJ161928
114	*Colletotrichum coccodes*	100	JN903942
115	*Aspergillus niger*	100	KF031027
119	*Epicoccum nigrum*	99	JX402182
120	*Gliocladium roseum*	99	AB470910

Table 2. Cont'd

ID	Microorganism	Homology (%)	Accession
121	*Ilyonectria macrodidyma*	99	JF807395
123	*Mortierella elongata*	99	FJ161922
124	*Penicillium digitatum*	100	HQ850932
126	*Fusarium tricinctum*	99	HM776425
127	*Fusarium solani*	99	JF912885
128	*Exophiala pisciphila*	99	DQ826739
129	*Rhizoctonia solani*	100	FJ746943
130	*Colletotrichum coccodes*	100	KC436058
131	*Trichoderma harzianum*	99	JQ617299
132	*Penicillium spinulosum*	100	JQ272447
134	*Penicillium polonicum*	100	KC692223
135	*Trichoderma harzianum*	100	KC576745
136	*Trichoderma harzianum*	99	JX173852
138	*Penicillium expansum*	100	KC009804
142	*Trichoderma harzianum*	100	JQ653078
143	*Penicillium melinii*	99	NR_077155
144	*Fusarium oxysporum*	100	KC254033
146	*Trichoderma asperellum*	100	KC576729
147	*Fusarium oxysporum*	100	JX853767
148	*Colletotrichum coccodes*	100	EF017205
149	*Pyrenochaeta lycopersici*	100	AY649583
150	*Fusarium oxysporum*	100	FJ158124
151	*Fusarium oxysporum*	100	JX897002
152	*Cladosporium cladosporioides*	100	HM776418
154	*Acremonium alternatum*	99	HE798150
158	*Botrytis aclada*	99	FJ169669
161	*Plectosphaerella cucumerina*	100	JX406529
162	*Thielaviopsis basicola*	99	KC305141
163	*Trichoderma atroviride*	100	FJ426394
164	*Aspergillus terreus*	100	KC113303

Table 2. Cont'd

ID	Microorganism	Homology (%)	Accession
165	*Aspergillus niger*	99	KC920839
166	*Aspergillus sydowii*	99	HQ637367
167	*Alternaria alternata*	100	JX406537
169	*Trichoderma viride*	100	HM037962
170	*Trichoderma harzianum*	100	JN179079
171	*Gliocladium roseum*	99	HQ637280
172	*Mucor fragilis*	99	GU566275
173	*Penicillium rugulosum*	100	KC797646
174	*Penicillium chrysogenum*	100	KC460873
175	*Penicillium citrinum*	100	HM486421
176	*Paecilomyces farinosus*	100	HQ907945
177	*Trichoderma viride*	100	JQ272443
178	*Aspergillus niger*	99	KC895529
179	*Aspergillus flavus*	100	JN226906
180	*Fusarium moniliforme*	99	JN672593
181	*Fusarium oxysporum*	100	KC577176
182	*Fusarium proliferatum*	100	JQ886420
183	*Fusarium oxysporum*	100	JN624906
184	*Cladosporium avellaneum*	99	EU040230
185	*Cladosporium* sp.	100	HF952644
186	*Fusarium culmorum*	99	AY147286
187	*Trichoderma pseudokoningii*	99	Z31014
188	*Cordyceps confragosa*	100	EU284719
189	*Phoma exigua*	100	GU395508
190	*Alternaria brassicicola*	100	JX648198
191	*Rhizoctonia solani*	100	JQ676881
192	*Rhizoctonia solani* AG-8	99	KC590586
194	*Alternaria tenuissima*	100	JX156349
ADAS-01	*Acremonium strictum*	99	FM998714
ADAS-02	*Chaetomium cochliodes*	99	KC460815
ADAS-03	*Cladosporium cladosporioides*	100	JX845290

Table 2. Cont'd

ID	Microorganism	Homology (%)	Accession
ADAS-05	*Penicillium chrysogenum*	100	HQ637353
ADAS-06	*Phytophthora cryptogea*	99	HM627524
ADAS-07	*Escherichia coli*	99	AP012306
ADAS-08	*Erwinia carotovora*	92	AF373187
ADAS-09	*Bacillus amyloliquefaciens*	100	HF563562
ADAS-10	*Xanthomonas campestris*	100	AE008922
ADAS-11	*Pseudomonas fluorescens*	93	CP003041
FERA-33	*Pythium sylvaticum*	96	AY598645
FERA-76	*Pythium violae*	100	HQ643965
FERA-91	*Chaetomium cochliodes*	100	JQ964802
FERA-130	*Alternaria solani*	100	AY154714
FERA-239	*Paecilomyces variotti*	100	AF033395
FERA-422	*Xanthomonas campestris* pv. *vesicatoria*	100	AF123088
FERA-757	*Phytophthora gonapodyides*	100	JX276089
FERA-881	*Xanthomonas gardneri*	100	AF123093
FERA-1431	*Xanthomonas campestris* pv. *raphani*	99	CP002789
FERA-1649	*Agrobacterium rhizogenes*	99	DQ682653
FERA-1822	*Alternaria tenuissima*	100	JQ989275
FERA-2038	*Alternaria alternata*	100	JX406537
FERA-2083	*Phytophthora palmivora*	100	FJ865408
FERA-2088	*Phytophthora parasitica*	99	JX418022
FERA-2407	*Agrobacterium tumefaciens*	93	AE007870
FERA-2547	*Cadophora gregata*	100	AB190396
FERA-2573	*Xanthomonas axonopodis*	100	AF123089
FERA-2577	*Pythium irregulare*	99	HQ643602
FERA-3214	*Ralstonia solanacearum*	100	FP885897
FERA-3264	*Clavibacter michiganensis* subsp. *michiganensis*	100	JN603295
FERA-4136	*Rhizobium* sp.	98	AF510923
FERA-4144	*Rhizobium tropici*	100	CP004015
FERA-4321	*Xanthomonas perforans*	100	GQ461740

The sequenced ITS/IGS regions of each organism being used to validate the array were then input into MEGA 5.1 and used to build a phylogenetic tree based on the homology of the ITS/IGS regions. The resulting tree was then separated into six groups of closely related organisms. A single organism was selected from each of the groups and run on the array in series allowing source of probe hybridisation to be inferred. Pooled groups of DNA were also run to allow comparative analysis of cross-hybridisation between single organism DNA and community DNA samples on the array.

Results and discussion

Fungi, oomycete and nematode probes

Due to the unavailability of some cultures 18 probes could not be validated and showed no activity during validation. These probes include; ARME, DOMI, EXXE, GIRO, MYRO, PALI, PEVA, PHCI, PRGO, PYAP, PYAR, PYDE, PYDI, PYEC, PYME, PYOL, PYTO and RHOR. Only two probes, MEIN for *Meloidogyne incognita* and CHSP *Chaetomium* spp. showed no activity when self-validated and may be non-functional.

Out of the remaining 52 fungal, oomycete and nematode specific probes 11 produced responses specific to their design with no cross-hybridisation. Eight of these were species-specific; CHCO, EXPI, FUSO2, PECH, PLCU2, RHSO, THBA2 and TRHA and three were genus specific; ALSP, PHOM and TRUN. Moreover, ALSO, PLCU1 and THBA1 showed a strong species-specific response with only ambiguous non-genera specific hybridisation. ASFL, ASTE, COCO1, AND VEDA all showed responses which favoured species specificity but also encountered a probability of some genus level interaction. All but VEDA also showed a possibility of low level inter-genera hybridisation. COAT, COCO2, FUOX2, FUSO1, GLRO and PYLY1 favoured their specific responses but showed increased levels of inter-genus cross hybridisation. ASSY, FUOX1, PHCR, PYIR, PYLY2, TRVI and VENI inferred that hybridisation of their specific species and closely related members within their genus may occur at equal rates, along with organisms outside of their genus. All but TRVI showed signs of probable inter-genera hybridisation. PEUN and CASP genus-specific probes showed specificity towards their respective genera but also probability towards a high level of cross hybridisation.

Seventeen of the probes that showed successful hybridisation were not self-validated. From the seventeen, eleven showed genus-specific hybridisation. Within the eleven ASUS, PHAR, PHNI, PYMY, and VEAL showed no probability of inter-genera cross hybridisation, however, FURE1,

FURE2, PEBR, PEGR and PYPA did. CLSP, DILY, GLIN, OLBR, PEAS, PRUN and SPSU, the remaining non self-validated probes, all showed non genera-specific responses.

Four probes designed for the identification of bacteria and one for nematodes showed cross-hybridisation when fungal and oomycete samples were run on the array. Three of these ERSP, YESP and NEUN, however, only produced ambiguous signals below the cut off value used during routine monitoring. CLMI and ECOL only produced low intensity signals.

Table 3. Inferred sources of hybridisation on fungal, oomycete and nematode probes and their signal intensity; ambiguous (±), strong (+) and very strong (++)

Probe	**Self-validated**	**Source of hybridisation**	
		Probable	**Possible**
ALSO	+	*Alternaria solani* (+)	*Thielaviopsis basicola* (+)
ALSP	+	*Alternaria* spp. (++)	
ASFL	+	*Aspergillus flavus* (++)	*Penicillium chrysogenum* (++)
		Aspergillus niger (+)	*Penicillium* spp. (++)
		Aspergillus terreus (+)	
ASSY	+	*Aspergillus sydowii* (++)	*Penicillium rugulosum* (++)
		Gliocladium roseum (++)	*Exophiala pisciphila* (+)
		Aspergillus niger (+)	*Fusarium moniliforme* (+)
		Plectosphaerella cucumerina (+)	*Fusarium solani* (+)
ASTE	+	*Aspergillus niger* (+)	
		Aspergillus terreus (++)	
ASUS	-	*Aspergillus sydowii* (+)	
CASP	+	*Cadophora gregata* (++)	*Exophiala pisciphila* (+)
		Verticillium spp. (+)	
CHCO	+	*Chaetomium cochliodes* (++)	

Table 3. Cont'd

Probe	Self-validated	Source of hybridisation	
		Probable	**Possible**
CHSP	+	*Botrytis cinerea* (++)	
		Gliocladium roseum (++)	
		Cordyceps confragosa (++)	
		Alternaria spp. (+)	
		Cladosporium spp. (+)	
		Fusarium spp. (+)	
		Plectosphaerella cucumerina (+)	
		Trichoderma spp. (+)	
CLSP	-	*Cladosporium* spp. (++)	*Exophiala pisciphila* (+)
		Aspergillus niger (+)	*Gliocladium roseum* (+)
		Verticillium spp. (+)	*Phytophthora* spp. (+)
COAT		*Colletotrichum acutatum* (++)	*Pyrenochaeta lycopersici* (+)
		Pythium spp. (+)	
		Verticillium spp. (+)	
COCO1	+	*Colletotrichum acutatum* (++)	*Fusarium tricinctum* (+)
		Colletotrichum coccodes (++)	*Trichoderma asperellum* (+)
			Trichoderma atroviride (+)
COCO2	+	*Colletotrichum coccodes* (+)	*Phoma exigua* (++)
		Epicoccum nigrum (+)	*Thielaviopsis basicola* (+)
DILY	-	*Phoma exigua* (+)	
EXPI	+	*Exophiala pisciphila* (++)	
FUOX1	+	*Fusarium* spp. (++)	*Phytophthora parasitica* (+)
		Gliocladium roseum (++)	*Pyrenochaeta lycopersici* (+)
		Aspergillus niger (+)	
		Ilyonectria spp. (+)	
		Pythium spp. (+)	
		Verticillium spp. (+)	

Table 3. Cont'd

Probe	Self-validated	Source of hybridisation	
		Probable	**Possible**
FUOX2	+	*Fusarium oxysporum* (++)	*Phytophthora palmivora* (+)
		Pythium spp. (+)	*Phytophthora parasitica* (+)
		Verticillium spp. (+)	*Pyrenochaeta lycopersici* (+)
			Trichoderma atroviride (+)
FURE1	-	*Fusarium* spp. (++)	*Aspergillus niger* (+)
		Gliocladium roseum (++)	*Phytophthora parasitica* (+)
		Ilyonectria spp. (+)	*Pyrenochaeta lycopersici* (+)
		Pythium spp. (+)	
		Verticillium spp. (+)	
FURE2	-	*Fusarium proliferatum* (+)	
		Ilyonectria macrodidyma (+)	
FUSO1	+	*Fusarium solani* (++)	
		Trichoderma harzianum (+)	
FUSO2	+	*Fusarium solani* (++)	
GLIN	-	-	*Alternaria brassicicola* (+)
			Fusarium culmorum (+)
GLRO	+	*Gliocladium roseum* (++)	*Aspergillus terreus* (+)
		Trichoderma harzianum (+)	
MEIN	+	-	
OLBR	-	*Penicillium* spp. (+)	*Botrytis cinerea* (+)
OOMY	+	*Phytophthora* spp. (++)	*Chaetomium cochliodes* (+)
		Pythium spp. (++)	*Cladosporium avellaneum* (++)
		Mortierella spp. (+)	*Exophiala pisciphila* (+)
PEAS	-	*Penicillium chrysogenum* (++)	*Thielaviopsis basicola* (+)
		Trichoderma viride (+)	*Trichoderma atroviride* (+)
PEBR	-	*Exophiala pisciphila* (+)	
		Penicillium chrysogenum (+)	
PECH	+	*Penicillium chrysogenum* (+)	
PEGR	-	*Penicillium citrinum* (++)	
		Aspergillus sydowii (+)	

Table 3. Cont'd

Probe	Self-validated	Source of hybridisation	
		Probable	**Possible**
PEUN	+	*Aspergillus* spp. (++)	*Thielaviopsis basicola* (+)
		Penicillium spp. (++)	*Trichoderma atroviride* (+)
		Trichoderma viride (+)	*Verticillium* spp. (+)
PHAR	-	*Phytophthora* spp. (+)	
PHCR	+	*Phytophthora* spp. (++)	*Pythium* spp. (+)
PHNI	-	*Phytophthora palmivora* (++)	
		Phytophthora parasitica (++)	
PHOM	+	*Phoma exigua* (+)	
PLCU1	+	*Plectosphaerella cucumerina* (++)	*Cadophora gregata* (+)
PLCU2	+	*Plectosphaerella cucumerina* (++)	
PRUN	-	-	*Phytophthora cryptogea* (+)
PYIR	+	*Pythium* spp. (++)	
		Phytophthora spp. (+)	
PYLY1	+	*Pyrenochaeta lycopersici* (++)	
		Alternaria spp. (+)	
PYLY2	+	*Alternaria solani* (++)	
		Pyrenochaeta lycopersici (++)	
PYMY	-	*Phytophthora* spp. (+)	
PYPA	-	*Pythium* spp. (++)	
		Phytophthora spp. (+)	
RHSO	+	*Rhizoctonia solani* (+)	
SPSU	-	*Phytophthora* spp. (+)	
		Pythium spp. (+)	
THBA1	+	*Thielaviopsis basicola* (++)	*Colletotrichum coccodes* (+)
THBA2	+	*Thielaviopsis basicola* (++)	
TRHA	+	*Trichoderma harzianum* (++)	
TRUN	+	*Trichoderma* spp. (++)	
TRVI	+	*Trichoderma asperellum* (++)	*Alternaria brassicicola* (+)
		Trichoderma atroviride (++)	*Aspergillus terreus* (+)
		Trichoderma viride (++)	
VEAL	-	*Verticillium dahlia* (++)	

Table 3. Cont'd

Probe	Self-validated	Source of hybridisation	
		Probable	**Possible**
VEDA	+	*Verticillium dahliae* (+)	
		Verticillium nigrescens (+)	
VENI	-	*Plectosphaerella cucumerina* (++)	
		Verticillium nigrescens (++)	

Bacterial probes

Fifteen bacterial strains were used to validate the array (see Table 1). The 15 strains produced 51 hybridisation signals in total. Thirty one of these were from probes designed to target bacteria but there were only four instances where the bacterial probes (ECOL, PSUN, RASO and XANT) were hybridised by their respective microorganisms. All other signals produced were due to cross-hybridisation. AGRH, BAAM, ERSP, PSSY, RHIZ and YESP showed no hybridisation during validation although all but PSSY and YESP were self-validated and may therefore be unreactive to the species used in validation.

Nineteen of the signals produced when running bacterial species on the array were from probes targeting fungi, oomycetes and nematodes. Eleven of these, however, were believed to be residual hybridised DNA from previous experiments and six were of ambiguous intensity. Only a single fungal probe GLIN showed a strong intensity with the addition of *Xanthomonas perforans* onto the array.

Table 4. Inferred sources of hybridisation on bacterial probes and their signals intensity; ambiguous (±), strong (+) and very strong (++)

Probe	Self-validated	Source hybridization	
		Probable	Possible
	+	-	-
AGRH	+	*Escherichia coli* (+)	
BAAM	+	-	-
BASU	+	-	*Escherichia coli* (±)
CLMI	+	*Agrobacterium rhizogenes* (+) *Rhizobium* spp. (+)	
ERSP	+	-	-
ECOL	+	*Escherichia coli* (+)	
NISP	-	*Agrobacterium rhizogenes* (+) *Clavibacter michiganensis* subsp. *michiganensis* (+)	*Agrobacterium tumefaciens* (+) *Rhizobium* spp. (±)
PSSY	-	-	-
PSUN	+	*Clavibacter michiganensis* subsp. *michiganensis* (+) *Bacillus amyloliquefaciens* (++)	*Rhizobium* spp. (+) *Pseudomonas fluorescens* (±)
RASO	+	*Ralstonia solanacearum* (+)	
RHIZ	+	-	-
XANT	+	*Agrobacterium rhizogenes* (+) *Clavibacter michiganensis* subsp. *michiganensis* (+)	*Rhizobium* spp. (+) *Rhizobium tropici* (±) *Xanthomonas perforans* (±)
YESP	-	-	-

Pooled DNA arrays

DNA from the microorganisms on two of the arrays used in series were pooled together and then run on the array. This allowed us to identify how samples containing DNA "communities" would react on the array. Both pooled arrays showed decreased levels of cross-hybridisation when compared to their non-community counterparts. The first array (array one) was validated with *Fusarium oxysporum* (FUOX1 and FUOX2), *Cladosporium cladosporiodes* (CLSP), *Paecilomyces farinosus*, *Mucor fragilis, Cadophora gregata* (CASP) and *Acremonium alternatum*

in series and then together on a pooled array. All non-target probes apart from CLMI showed reduced levels of hybridisation including PEUN, PEAS, PLCU1.

Array two was run with *Fusarium oxysporum* (FUOX1 and FUOX2)*, Colletotrichum acutatum* (COAT)*, Penicillium rugulosum* (PEUN)*, Botrytis aclada, Mortierella elongate and Trichoderma viride* (TRVI). Again, array two showed improved levels of non-specific hybridisation for the pooled array. Reductions in hybridisation to the non-target probes ALSO, ASSY, CESP, COCO, PYLY1 were observed. The non-target probes FURE1 and PEAS showed no reduction in hybridisation on the pooled array.

Table 5. Hybridisation patterns from arrays run with pooled and successive addition of DNA and their signals intensity; ambiguous (±), strong (+) and very strong (++)

Array 1			Array 2		
Probe	Pooled	Series	Probe	Pooled	Series
CASP	++	++	ALSO	±	+
CLMI	±	-	ASSY	+	++
CLSP	+	++	CESP	-	±
FUOX1	±	++	COAT	++	++
FUOX2	++	+	COCO	+	++
PEUN	-	+	FUOX1	+	++
PEAS	-	++	FUOX2	++	++
PLCU1	-	+	FURE1	+	+
			PEUN	+	++
			PEAS	+	+
			PYLY1	-	+
			TRUN	++	++
			TRVI	++	++

The results of microarray validation tests for individual species and genera are summarised in Table 6.

Table 6. Summary of microarray validation tests

Microorganism species/genus	Type[a]	Self-validated	Cross-hybridisation[b]		
			None	Genus level	Inter-genus
Pathogens					
Alternaria solani	F	+			(+)
Armillaria mellea	F	NT			
Colletotrichum acutatum	F	+			(+)
*Colletotrichum coccodes**	F	+		(++)	(++)
Didymella lycopersici	F	NT			(+)
*Fusarium oxysporum**	F	+		(+)	(+)
*Fusarium redolens**	F	NT		(++)	(++)
*Fusarium solani**	F	+			(+)
Phytophthora arecae	O	NT		(+)	
Phytophthora cinnamomi	O	NT			
Phytophthora cryptogea	O	+		(++)	(+)
Phytophthora nicotianae	O	NT		(++)	
*Plectosphaerella cucumerina**	F	+	+		
*Pyrenochaeta lycopersici**	F	+			(++)
Pythium aphanidermatum	O	NT			
Pythium arrhenomones	O	NT			
Pythium debaryum	O	NT			
Pythium diclinum	O	NT			
Pythium echinulatum	O	NT			
Pythium irregulare	O	+		(++)	(+)
Pythium megalacanthum	O	NT			
Pythium myriotylum	O	NT			(+)
Pythium oligandrum	O	NT			
Pythium paroencandrum	O	NT		(++)	(+)
Pythium torulosum	O	NT			
Rhizoctonia solani	F	+	+		
Spongospora subterranea	F	NT			(+)
*Thielaviopsis basicola**	F	+			(+)
Verticillium albo-atrum	F	NT		(++)	
Verticillium dahliae	F	+		(+)	
Verticillium nigrescens	F	+			(++)

Table 6. Cont'd

Saprophytes					
Alternaria spp.	F	+	+		
Aspergillus flavus	F	+		(+)	(++)
Aspergillus sydowii	F	+		(+)	(++)
Aspergillus terreus	F	+		(++)	
Aspergillus ustus	F	NT		(+)	
Cadophora spp.	F	+			(+)
Cephalosporium spp.	F	+			(±)
Chaetomium cochliodes	F	+	+		
Chaetomium spp.	F	+			(++)
Cladosporium spp.	-	NT			(++)
Doratomyces microsporus	F	NT			
Exophiala pisciphila	F	+	+		
Exophiala xenobiotica	F	NT			
Gigaspora rosea	F	NT			
Gliocladium roseum	F	+			(+)
Glomus intraradices	F	NT			(+)
Myrothecium roridum	F	NT			
Olpidium brassicae	F	NT			(+)
Paecilomyces lilacinus	F	NT			
Penicillium brevicompactum	F	NT		(+)	(+)
Pencillium chrysogenum	F	+	+		
Penicillium griseofulvum	F	NT		(++)	(+)
Pencillium variabile	F	NT			
Penicillium spp.	F	+			(++)
Petriella asymmetrica	F	NT			(++)
Phoma sp.	F	+	+		
Rhizopus oryzae	F	NT			
Trichoderma viride	F	+		(++)	(+)
Trichoderma harzianum	F	+	+		
Trichoderma spp.	F	+	+		
Bacteria					
Agrobacterium rhizogenes		+			(+)
Agrobacterium tumefaciens		+			

Table 6. Cont'd

Bacillus amyloliquefaciens	+		
Bacillus subtilis	+		(+)
Clavibacter sp.	+		(+)
Erwinia spp.	+		
Escherichia coli	+	+	
Nitrospira spp. (nitrite oxidising bacterium)	NT		(+)
Pseudomonas syringae	NT		
Pseudomonas universal	+		(++)
Ralstonia solanacearum	+	+	
Rhizobium sp.	+		
Xanthomonas spp.	+		(+)
Yersinia spp.	NT		
Nematodes			
Meloidogyne incognita	+		
Pratylenchus goodeyi	NT		
Pratylenchus spp.	NT		(+)

[a] F – fungus: O – oomycete

[b] signal intensity; (±) ambiguous; (+) strong; (++) very strong.

NT – not tested

*represents data from two probes

Objective 2 – Monitor rhizosphere microorganisms over a season

Introduction

Information on the occurrence and relative abundance of microorganisms in the rhizosphere is potentially useful to assess tomato root health. In order to gain insight on possible microbial indicators of good and poor root health, it is first necessary to describe the microbial populations normally found on roots and whether this changes with crop age and irrigation system (including method of treating recycled water). The aim of this work was (1) to monitor microorganism populations on roots of tomato grown in rockwool over a growing season; (2) to compare the populations on roots in three contrasting irrigation systems; (3) to compare the populations on roots with two contrasting water treatment systems.

Materials and methods

Site and crop details

Routine monitoring/Comparison of irrigation systems

Monitoring was done on three commercial crops from weeks 2 to week 42. The sites were selected so that the crops were all grown on rockwool slabs and were all on the same rootstock. The irrigation systems were run-to-waste, recycling with part of the water passed through a slow sand filter (SSF) and recycling with all of the water passed through a SSF (Table 7). Associated information on crop production is given in Table 8 and trial diaries are given in Appendix 1.

Comparison of water treatment systems

Monitoring was done on four occasions (once every 2 months) during 2013 on two commercial crops. Both were grown on rockwool slabs and used the same rootstock. The recycled water was treated by a UV system or a pasteuriser (Table 7). Associated crop production information is given in Table 8 and trial diaries are given in Appendix 2.

Root and solution sampling

Samples were taken from a row towards the middle of a block. On each occasion four adjacent slabs were sampled. The four adjacent slabs were used at the next sampling, and so on, so that each slab was sampled once only. A small portion of young roots was collected from the bottom of each slab (samples I to IV), except at the first sampling when roots were taken from the base and sides of four cubes; and the second sampling when roots were taken from the slab directly beneath the cube.

A bulk sample of irrigation solution (c. 20 ml) was collected from the four sampled slabs using a sterile plastic syringe. Samples were posted to Nottingham University where they were assessed for discolouration and rot and then processed to extract the DNA. The DNA was tested on the microarray immediately, or frozen if this was not possible.

Array intensity values were classified as nil (0), low (1), medium (2) or high (3). The values for the four replicate samples were added together resulting in a scale of 0 (not detected) to 12 (present at high levels in all samples).

Assessments

Root assessment

At the time of sampling the roots in each slab were assessed on a 1-5 scale:

1 – Very poor roots. Very few white root tips; obvious rotting

2 – Poor roots. Some white roots but obvious rot or discolouration

3 – Moderate roots. Moderate extent of white roots; some rot or discolouration

4 – Good roots. Ample white roots; trace of rot or discolouration

5 – Excellent roots. Extensive dense white root tips; no rotting or discolouration.

Roots were also scored on receipt at Nottingham University using the same scale.

Stem base

At the time of each root sampling, stem bases were also examined and assessed for:

- Number of green stem bases (healthy plants)
- Number of yellowing/brown stem bases (dying plants)
- Number of dead and missing stem bases

Assessments of all sampled plants were done in August and October. In addition to the root and stem base assessments described above, plants were examined for symptoms of root mat disease, Verticillium and Fusarium wilt diseases, adventitious rooting on the stem, and vascular staining in the stem bases of the scion crop. Vascular staining was assessed on a 0-3 scale (nil, pale brown, medium brown, dark brown) on all plants at the final assessment only, just prior to the reduction of slab irrigation.

Table 7. Detail of tomato crops monitored for root microorganisms – 2013

Site	Irrigation system	Water treatment (abbreviation)	Rootstock	Number sample occasions
Routine monitoring/Comparison of irrigation systems				
1. Nursery A, West Sussex	Run-to-waste	Nil (RTW)	Maxifort	14
2. Nursery B, Norfolk	Re-cycled (open lagoon)	Part treated by SSF (pSSF)	Maxifort	20
3. Nursery C, Yorkshire	Re-cycled (covered tanks)	All treated by SSF (fSSF)	Maxifort	16
Comparison of water treatment systems				
4. Nursery D, Yorkshire	Re-cycled	UV	Emperador	4
5. Nursery E, Yorkshire	Re-cycled	Heat	Emperador	4

Table 8. Supplementary information on crops monitored for root microorganisms - 2013

Site	Original water source	Slab type	Scion variety	Other water treatment	Number/slab	
					Cubes	Stem bases
Routine monitoring						
1. Nursery A West Sussex	Borehole	Cultilene Optimax	Mecano	None	2	4
2. Nursery B Norfolk	Roof water (via reservoir)	Cultilene Exact	Sweetelle	Reciclean (from 6 Feb) Ultrasound in day tank (from 6 Feb)	2	4
3. Nursery C Yorkshire	Borehole	Cultilene Maxima X - fibre	Garincha	None	3	6
Periodic monitoring						
4. Nursery D Yorkshire (UV)	Borehole	Grotop	Dometica	UV light	2	4
5. Nursery E Yorkshire (heat)	Borehole	Grodan Expert	Dometica	Heat treatment	2	4

Results and discussion

Objective 2a – Monitor rhizosphere microorganisms over a season

Results for individual nurseries (A-E) are presented in Table 9-13. Table 16 lists and compares the microorganisms found at least once on the five nurseries. Table 15 lists the microorganisms found in each of the five crops and Table 16 lists the microorganisms not detected in any of the crops. Table 17 compares microbial diversity at selected sample times. Table 18 lists the microorganisms present in planting cubes and rockwool slab solution prior to the slab contact.

The primer sequences used in the array were each designed for a particular microbial species or genus known to occur on tomato roots. It is possible that the same sequence as used for one of the microarray targets may occur in a different microorganism resulting in a cross-reaction (i.e. a false positive result). The greatest level of confidence can be given in the microarray tests to those primers which were a) self-validated and b) did not show a very strong cross-reaction. These were:

Pathogens	Saprophytes	Bacteria
Alternaria solani	***Alternaria* spp.**	*Agrobacterium rhizogenes*
Aspergillus flavus		
Aspergillus terreus		
Colletotrichum coccodes	*Cadophora* spp.	
	Chaetomium cochliodes	*Bacillus amyloliquefaciens*
Fusarium solani	*Fusarium oxysporum*	*Bacillus subtilis*
Plectosphaerella cucumerina	***Exophiala pisciphila***	
Rhizoctonia solani	*Gliocladium roseum*	*Erwinia* sp.
Thielaviopsis basicola	***Penicillium chrysogenum***	
Verticillium dahliae	***Phoma* spp.**	***Ralstonia solanacearum***
	Trichoderma harzianum	*Rhizobium* sp.
	***Trichoderma* spp.**	

Names in bold showed no cross-reaction.

Results presented in this section should therefore be interpreted with a degree of caution in mind.

Table 9a. Occurrence of fungi, bacteria and nematodes on tomato roots of cv. Maxifort in a crop grown on rockwool slabs with the irrigation water run-to-waste - weeks 4-26, 2013 (nursery A), as detected by microarray

Microorganism	Relative abundance (0-12) by week number							
	4	8	10	14	16	20	24	26
Pathogenic fungi								
Colletotrichum acutatum	0	0	0	2	2	0	0	0
Colletotrichum coccodes	0	0	0	0	0	0	0	0
Fusarium oxysporum	10	10	12	8	8	4	0	1
Fusarium redolens	8	6	10	5	4	2	0	0
Pythium diclinum	0	0	2	0	1	1	0	0
Pythium irregulare	5	1	3	3	4	3	1	2
Pythium megalacanthum	0	0	0	0	0	0	0	0
Pythium myriotylum	1	0	3	0	2	2	0	0
Pythium paroencandrum	6	3	5	3	2	3	0	2
Pythium torulosum	0	0	0	0	0	0	0	0
Plectosphaerella cucumerina	12	9	12	12	11	8	5	7
Pyrenochaeta lycopersici	0	0	0	0	0	1	0	1
Spongospora subterranea	4	0	0	1	1	3	0	1
Verticillium nigrescens	11	8	12	11	10	6	3	7
Saprophytic fungi								
Aspergillus flavus	4	2	7	7	5	4	0	1
Aspergillus terreus	0	0	0	0	0	0	0	0
Aspergillus ustus	0	0	0	1	1	3	0	1
Cadophora spp.	0	0	0	0	0	0	0	0
Cladosporium spp.	6	9	10	7	4	6	1	2
Myrothecium roridum	0	0	0	0	0	0	0	0
Olpidium brassicae	0	0	0	0	0	0	0	0
Penicillium brevicompactum	2	1	2	3	1	1	0	0
Penicillium spp.	6	5	10	8	6	7	1	4
Petriella asymmetrica	4	2	4	5	4	4	0	1
Phoma spp.	0	0	0	0	0	0	0	0
Trichoderma viride	0	0	0	0	0	0	0	0
Bacteria and nematodes								
Agrobacterium rhizogenes	0	6	4	3	5	4	0	0
Agrobacterium tumefaciens	0	1	0	0	0	0	0	0

Table 9a. Cont'd

Microorganism	Relative abundance (0-12) by week number							
	4	8	10	14	16	20	24	26
Erwinia spp.	8	10	8	3	0	1	0	1
Nitrospira spp.	11	12	12	12	12	10	8	12
Pseudomonas (universal)	12	12	12	12	12	11	12	12
Xanthomonas spp.	8	9	8	10	10	8	5	7
Pratylenchus spp.	0	2	3	1	1	0	0	2
Universal probes								
Bacteria	12	12	12	12	12	10	8	12
Fungi	12	9	12	12	11	9	9	8
Nematode	8	3	3	5	3	3	0	1
Oomycete	7	3	6	10	7	6	4	3

Table 9b. Occurrence of fungi, bacteria and nematodes on tomato roots of cv. Maxifort in a crop grown on rockwool slabs with the irrigation water run-to-waste - weeks 28-42, 2013 (nursery A), as detected by microarray

Microorganism	Relative abundance (0-12) by week number					
	28	30	31	33	35	38
Pathogenic fungi						
Colletotrichum acutatum	0	0	0	0	0	0
Colletotrichum coccodes	0	0	0	0	0	0
Fusarium oxysporum	2	2	2	2	0	1
Fusarium redolens	1	0	1	0	0	0
Pythium diclinum	0	0	1	2	3	2
Pythium irregulare	2	5	5	4	3	2
Pythium megalacanthum	0	0	0	0	0	0
Pythium myriotylum	0	0	2	3	6	3
Pythium paroencandrum	0	1	2	1	3	0
Pythium torulosum	0	0	0	0	0	0
Plectosphaerella cucumerina	10	9	9	10	7	8
Pyrenochaeta lycopersici	0	0	0	0	0	0
Spongospora subterranea	4	3	2	3	5	0
Verticillium nigrescens	7	8	6	6	4	5
Saprophytic fungi						

Table 9b. Cont'd

Aspergillus flavus	0	2	1	0	0	0
Aspergillus terreus	0	0	2	0	0	0
Aspergillus ustus	4	0	0	0	0	0
Cadophora spp.	0	0	0	0	0	0
Cladosporium spp.	5	4	3	1	0	0
Myrothecium roridum	0	0	0	0	0	0
Olpidium brassicae	0	0	0	0	1	3
Penicillium brevicompactum	0	0	0	0	0	0
Penicillium spp.	2	3	2	1	0	0
Petriella asymmetrica	0	2	1	0	0	0
Phoma spp.	0	0	0	0	0	0
Trichoderma viride	0	0	0	0	0	0
Bacteria and nematodes						
Agrobacterium rhizogenes	0	1	1	1	1	0
Agrobacterium tumefaciens	0	0	0	0	0	0
Erwinia spp.	0	0	0	0	1	0
Nitrospira spp.	12	10	12	11	10	8
Pseudomonas (universal)	12	12	12	12	12	9
Xanthomonas spp.	7	7	7	8	6	1
Pratylenchus spp.	1	1	0	1	0	0
Universal probes						
Bacteria	11	9	10	11	11	8
Fungi	11	11	12	10	9	9
Nematode	0	1	0	3	2	2
Oomycete	3	4	10	6	6	6

Table 10a. Occurrence of fungi, bacteria and nematodes on tomato roots, cv. Maxifort, in a crop grown on rockwool slabs and with the irrigation water recycled and part of it treated by a slow sand filter - weeks 2-26, 2013 (nursery B) as detected by microarray

Microorganism	Relative abundance (0-12) by week number												
	2	4	6	8	10	12	14	16	18	20	22	24	26
Pathogenic fungi													
Colletotrichum acutatum	0	0	0	0	0	0	6	1	0	0	0	0	0
Colletotrichum coccodes	0	0	0	0	0	0	0	0	0	2	5	3	1
Fusarium oxysporum	8	5	5	11	10	10	8	5	8	1	6	4	1
Fusarium redolens	2	3	4	4	8	6	5	1	6	0	3	2	1
Fusarium solani	0	0	0	0	0	0	0	0	0	0	0	0	0
Phytophthora cryptogea	0	0	0	0	0	0	0	0	1	0	0	0	0
Pythium diclinum	1	0	0	0	0	1	1	2	7	4	3	3	7
Pythium irregulare	2	3	0	0	0	4	3	4	6	3	4	1	3
Pythium megalacanthum	1	0	0	0	0	0	0	0	0	0	0	0	0
Pythium myriotylum	1	0	0	0	0	1	2	2	9	6	4	5	7
Pythium paroencandrum	2	8	0	1	2	0	0	2	4	1	1	0	0
Pythium torulosum	2	0	0	0	0	0	0	0	0	0	0	0	0
Plectosphaerella cucumerina	5	6	7	12	9	12	12	10	12	12	11	10	12
Pyrenochaeta lycopersici	0	0	0	0	0	0	0	0	0	0	0	0	1
Rhizoctonia solani	0	0	0	0	1	1	0	1	1	2	1	0	0
Spongospora subterranea	0	4	0	0	1	0	0	0	4	1	4	0	1
Verticillium albo-atrum	0	0	0	0	0	0	0	2	0	1	0	0	0
Verticillium nigrescens	6	4	5	11	7	10	11	7	10	10	7	7	10
Saprophytic fungi													
Aspergillus flavus	9	5	4	2	5	3	5	4	7	7	3	0	4
Aspergillus ustus	2	0	0	0	0	1	4	0	0	0	0	0	0
Cadophora spp.	1	0	0	0	0	0	0	0	0	0	0	0	0
Cladosporium spp.	11	2	3	6	5	3	3	6	7	7	1	4	7
Myrothecium roridum	4	0	0	0	0	0	0	0	0	0	0	0	0
Olpidium brassicae	2	8	6	7	10	8	7	6	7	5	3	6	6
Penicillium brevicompactum	5	0	2	1	1	0	0	1	3	3	1	0	0
Penicillium variabile	0	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium spp.	11	7	6	3	8	5	8	7	10	8	2	1	8
Petriella asymmetrica	8	3	4	1	2	2	8	3	4	4	2	0	0
Phoma spp.	3	0	0	0	0	1	0	0	0	0	0	0	0

Table 10a. Cont'd

Microorganism	Relative abundance (0-12) by week number												
	2	4	6	8	10	12	14	16	18	20	22	24	26
Trichoderma viride	2	0	0	0	0	0	0	0	0	0	0	0	0
Bacteria and nematodes													
Agrobacterium rhizogenes	4	4	1	2	2	0	0	1	1	0	0	0	1
Agrobacterium tumefaciens	2	1	0	2	0	1	1	1	1	0	0	1	0
Bacillus amyloliquefaciens	0	0	0	0	3	0	0	1	0	0	0	0	0
Bacillus subtilis	0	0	0	0	3	0	0	1	0	0	0	0	0
Clavibacter sp.	0	0	0	0	0	0	0	0	2	0	0	0	0
Erwinia spp.	7	11	11	10	10	7	3	1	3	0	1	3	1
Nitrospira spp.	8	12	11	11	12	12	12	11	12	11	8	10	12
Pseudomonas (universal)	12	12	11	12	12	12	12	12	12	11	12	10	12
Xanthomonas spp.	8	11	6	8	10	8	8	10	9	7	4	5	7
Pratylenchus spp.	2	3	0	1	1	4	1	2	2	2	0	3	2
Universal probes													
Bacteria	10	12	11	12	12	12	12	12	12	11	8	10	12
Fungi	12	12	11	12	12	12	12	12	12	12	12	12	12
Nematode	10	0	0	6	8	7	9	7	8	2	0	5	6
Oomycete	12	5	5	5	5	7	8	7	8	9	5	6	11

Recycling of irrigation water started in week 10.

Table 10b. Occurrence of fungi, bacteria and nematodes on tomato roots, cv. Maxifort, in a crop grown on rockwool slabs and with the irrigation water recycled and part of it treated by a slow sand filter - weeks 28-40, 2013 (nursery B) as detected by microarray

Microorganism	Relative abundance (0-12) by week number						
	28	30	32	34	36	38	40
Pathogenic fungi							
Colletotrichum acutatum	1	0	0	0	0	0	0
Colletotrichum coccodes	3	1	0	9	6	3	1
Fusarium oxysporum	5	0	2	8	7	3	0
Fusarium redolens	3	0	0	6	3	2	0
Fusarium solani	0	0	0	1	0	0	0
Phytophthora cryptogea	0	0	0	2	0	0	0
Pythium diclinum	3	2	3	5	5	2	1
Pythium irregulare	2	5	1	7	6	1	2
Pythium megalacanthum	0	0	0	0	0	0	0
Pythium myriotylum	3	2	5	8	7	3	2
Pythium paroencandrum	0	1	2	3	4	0	0
Pythium torulosum	0	0	0	0	0	0	0
Plectosphaerella cucumerina	8	5	7	11	10	4	5
Pyrenochaeta lycopersici	0	0	1	1	3	0	0
Rhizoctonia solani	0	0	0	0	0	0	0
Spongospora subterranea	0	2	0	7	6	0	0
Verticillium albo-atrum	0	0	0	0	2	0	0
Verticillium nigrescens	7	4	5	7	8	2	4
Saprophytic fungi							
Aspergillus flavus	2	1	3	1	5	0	0
Aspergillus ustus	0	0	0	0	0	0	0
Cadophora spp.	0	1	0	0	0	0	0
Cladosporium spp.	1	0	6	6	6	0	2
Myrothecium roridum	0	0	0	0	0	0	0
Olpidium brassicae	7	4	3	3	2	0	0
Penicillium brevicompactum	1	0	0	0	0	0	0
Penicillium variabile	0	0	1	1	0	0	0
Penicillium spp.	2	3	6	3	9	0	1

Table 10b. Cont'd

Microorganism	Relative abundance (0-12) by week number						
	28	30	32	34	36	38	40
Petriella asymmetrica	1	0	2	0	0	0	0
Phoma spp.	0	0	0	0	0	0	0
Trichoderma viride	0	0	0	0	0	0	0
Bacteria and nematodes							
Agrobacterium rhizogenes	0	0	0	1	1	0	0
Agrobacterium tumefaciens	0	0	0	0	0	0	0
Bacillus amyloliquefaciens	0	0	0	0	1	0	0
Bacillus subtilis	0	0	0	0	1	0	0
Clavibacter sp.	0	0	0	0	0	0	0
Erwinia spp.	4	2	2	2	2	0	0
Nitrospira spp.	9	7	12	12	11	7	5
Pseudomonas (universal)	9	8	12	12	12	8	5
Xanthomonas spp.	4	4	9	7	8	4	3
Pratylenchus spp.	2	0	1	1	0	0	0
Universal probes							
Bacteria	9	7	12	12	12	7	5
Fungi	12	9	12	12	11	8	9
Nematode	4	2	1	6	6	4	1
Oomycete	7	3	5	8	8	7	8

Recycling of irrigation water started in week 10.

Table 11a. Occurrence of fungi, bacteria and nematodes on tomato roots cv. Maxifort in a crop grown on rockwool slabs with the irrigation water all recycled through a SSF - weeks 4-26, 2013 (nursery C), as detected by microarray

Microorganism	Relative abundance (0-12) by week number									
	4	8	10	13	16	18	20	22	24	26
Pathogenic fungi										
Colletotrichum acutatum	0	0	0	0	2	0	0	0	0	0
Colletotrichum coccodes	1	0	0	0	0	0	0	0	0	0
Fusarium oxysporum	9	5	7	3	6	4	6	3	6	2
Fusarium redolens	5	3	6	1	4	1	3	1	3	0
Phytophthora arecae	0	0	0	0	0	0	0	0	0	0
Pythium diclinum	0	0	0	0	0	2	6	6	0	0
Pythium irregulare	8	0	0	3	5	2	2	8	2	5
Pythium megalacanthum	0	1	0	0	0	0	0	0	0	0
Pythium myriotylum	0	0	0	0	0	2	7	7	0	0
Pythium paroencandrum	8	2	0	3	1	2	0	2	0	2
Pythium torulosum	0	0	0	0	0	0	0	0	0	0
Plectosphaerella cucumerina	4	0	3	6	12	9	12	12	11	12
Pyrenochaeta lycopersici	0	0	0	0	0	0	0	0	0	1
Spongospora subterranea	0	0	0	0	2	2	0	5	0	6
Verticillium albo-atrum	0	0	0	3	2	3	4	4	4	2
Verticillium dahliae	0	0	0	0	0	0	0	0	0	0
Verticillium nigrescens	11	0	5	9	12	11	11	11	11	12
Saprophytic fungi										
Aspergillus flavus	8	4	10	7	9	10	11	9	7	8
Aspergillus ustus	0	0	0	0	0	0	0	0	0	0
Cadophora spp.	0	0	0	0	0	0	0	0	2	0
Cephalosporium spp.	0	0	0	0	0	0	0	0	0	1
Cladosporium spp.	11	10	11	9	11	10	10	9	10	11
Exophiala pisciphila	0	0	0	0	0	0	0	0	0	0
Myrothecium roridum	0	0	0	0	0	0	0	0	0	0
Olpidium brassicae	6	0	0	1	0	0	0	1	0	0
Penicillium brevicompactum	4	1	8	3	5	5	8	5	4	6
Penicillium variabile	0	0	0	0	0	0	0	1	0	0
Penicillium spp.	9	6	11	9	10	11	9	10	11	11

Table 11a. Cont'd

Microorganism	Relative abundance (0-12) by week number									
	4	8	10	13	16	18	20	22	24	26
Petriella asymmetrica	5	3	9	6	8	7	10	7	6	7
Phoma spp.	0	0	0	0	0	0	0	0	0	0
Trichoderma viride	0	0	0	0	0	0	0	0	0	0
Bacteria and nematodes										
Agrobacterium rhizogenes	4	1	0	2	3	1	0	2	0	2
Agrobacterium tumefaciens	0	0	0	0	0	0	0	0	0	0
Bacillus amyloliquefaciens	1	0	0	0	0	0	1	0	0	0
Bacillus subtilis	0	0	0	0	0	0	1	0	0	0
Clavibacter sp.	2	0	0	1	2	1	3	3	0	4
Erwinia spp.	6	9	2	2	0	0	6	0	0	5
Nitrospira spp.	12	10	12	12	12	12	12	12	11	12
Pseudomonas (universal)	12	12	12	12	12	12	12	12	12	12
Rhizobium spp.	0	0	0	0	0	0	0	0	0	0
Xanthomonas spp.	7	8	10	10	8	8	9	8	5	6
Pratylenchus spp.	0	0	1	0	1	2	0	2	0	2
Universal probes										
Bacteria	12	11	12	12	12	12	12	12	12	12
Fungi	12	9	12	11	12	12	12	12	11	12
Nematode	5	3	3	10	12	9	5	6	5	0
Oomycete	7	3	6	7	9	8	10	9	8	4

Recycling of irrigation water started in week 10.

Table 11b. Occurrence of fungi, bacteria and nematodes on tomato roots cv. Maxifort in a crop grown on rockwool slabs with the irrigation water all recycled through a SSF – weeks 28-40, 2013 (nursery C), as detected by microarray

Microorganism	Relative abundance (0-12) by week number					
	28	30	34	36	38	40
Pathogenic fungi						
Colletotrichum acutatum	0	0	0	0	0	0
Colletotrichum coccodes	0	0	0	2	2	3
Fusarium oxysporum	2	4	2	3	3	0
Fusarium redolens	0	3	1	2	1	0
Phytophthora arecae	0	0	0	0	1	0
Pythium diclinum	4	8	8	3	6	8
Pythium irregulare	5	9	2	9	8	7
Pythium megalacanthum	0	0	0	0	0	0
Pythium myriotylum	5	10	9	6	8	8
Pythium paroencandrum	3	3	0	5	3	0
Pythium torulosum	0	0	0	0	0	0
Plectosphaerella cucumerina	11	10	10	8	8	11
Pyrenochaeta lycopersici	1	0	0	1	1	0
Spongospora subterranea	6	6	1	6	7	4
Verticillium albo-atrum	5	4	4	1	4	1
Verticillium dahliae	0	1	0	0	0	0
Verticillium nigrescens	10	11	9	5	9	10
Saprophytic fungi						
Aspergillus flavus	10	10	4	4	5	8
Aspergillus ustus	0	0	0	0	0	0
Cadophora spp.	0	0	0	0	0	0
Cephalosporium spp.	0	1	0	0	0	0
Cladosporium spp.	12	8	8	7	9	10
Exophiala pisciphila	0	0	0	3	2	1
Myrothecium roridum	0	0	0	0	0	0
Olpidium brassicae	0	0	1	1	2	0
Penicillium brevicompactum	6	2	1	2	1	4
Penicillium variabile	3	3	0	3	0	1
Penicillium spp.	11	11	7	6	6	8

Table 11b. Cont'd

Microorganism	Relative abundance (0-12) by week number					
	28	30	34	36	38	40
Petriella asymmetrica	8	7	3	3	1	4
Phoma spp.	0	0	0	0	0	0
Trichoderma viride	0	0	0	0	0	0
Bacteria and nematodes						
Agrobacterium rhizogenes	1	0	0	1	3	3
Agrobacterium tumefaciens	0	0	0	0	0	0
Bacillus amyloliquefaciens	0	0	0	0	0	0
Bacillus subtilis	0	0	0	0	0	0
Clavibacter sp.	2	3	0	3	0	3
Erwinia spp.	2	4	2	2	4	3
Nitrospira spp.	11	11	10	9	11	12
Pseudomonas (universal)	11	11	12	9	12	12
Rhizobium spp.	0	1	0	0	1	0
Xanthomonas spp.	8	8	7	5	8	8
Pratylenchus spp.	2	1	0	5	7	5
Universal probes						
Bacteria	11	11	10	9	11	12
Fungi	12	12	10	10	12	11
Nematode	8	5	3	7	7	11
Oomycete	10	11	10	11	10	9

Recycling of irrigation water started in week 10.

Table 12. Occurrence of fungi, bacteria and nematodes on tomato roots of cv. Emperador grown on rockwool slabs with the irrigation water all recycled through a UV treatment system (nursery D), as detected by microarray

Microorganism	Relative abundance by week number			
	8	16	26	38
Pathogenic fungi				
Colletotrichum acutatum	0	1	0	0
Colletotrichum coccodes	0	0	2	2
Fusarium oxysporum	4	5	3	0
Fusarium redolens	2	1	1	0
Pythium diclinum	0	2	4	2
Pythium irregulare	3	1	8	8
Pythium megalacanthum	0	0	0	0
Pythium myriotylum	0	2	5	3
Pythium paroencandrum	2	0	2	0
Pythium torulosum	0	0	0	0
Plectosphaerella cucumerina	5	12	8	6
Pyrenochaeta lycopersici	0	0	0	1
Spongospora subterranea	0	0	4	2
Verticillium nigrescens	3	10	6	4
Saprophytic fungi				
Aspergillus flavus	1	7	2	1
Aspergillus ustus	0	0	0	0
Cadophora spp.	0	0	0	0
Cladosporium spp.	0	3	3	3
Myrothecium roridum	0	0	0	0
Olpidium brassicae	0	0	2	0
Penicillium brevicompactum	0	3	0	0
Penicillium spp.	4	9	3	2
Petriella asymmetrica	1	5	0	0
Phoma spp.	0	0	0	0
Trichoderma viride	0	0	0	0
Trichoderma spp.	0	0	0	1
Bacteria and nematodes				
Agrobacterium rhizogenes	2	1	0	0
Agrobacterium tumefaciens	0	0	0	0

Table 12. Cont'd

Microorganism	Relative abundance by week number			
	8	16	26	38
Clavibacter sp.	0	0	1	0
Erwinia spp.	12	8	9	0
Nitrospira spp.	12	12	11	8
Pseudomonas (universal)	12	12	12	8
Xanthomonas spp.	9	10	8	4
Pratylenchus spp.	0	1	3	0
Universal probes				
Bacteria	12	12	11	8
Fungi	9	12	10	8
Nematode	8	6	2	6
Oomycete	1	6	6	8

Table 13. Occurrence of fungi, bacteria and nematodes on tomato roots of cv. Emperador grown on rockwool slabs with the irrigation water all recycled through a heat-treatment system (nursery E), a detected by microarray

Microorganism	Relative abundance by week number			
	8	16	26	38
Pathogenic fungi				
Colletotrichum acutatum	0	2	0	0
Colletotrichum coccodes	0	3	7	7
Fusarium oxysporum	2	5	1	0
Fusarium redolens	1	3	2	2
Pythium diclinum	0	0	2	2
Pythium irregulare	0	1	6	2
Pythium megalacanthum	0	0	0	0
Pythium myriotylum	0	0	2	2
Pythium paroencandrum	2	0	3	0
Pythium torulosum	0	0	0	0
Plectosphaerella cucumerina	3	10	12	7
Rhizoctonia solani	1	0	1	0
Spongospora subterranea	0	0	7	2

Table 13. Cont'd

Microorganism	Relative abundance (0-12) by week number			
	8	16	26	38
Verticillium nigrescens	2	9	9	5
Saprophytic fungi				
Aspergillus flavus	0	3	6	3
Aspergillus ustus	0	0	0	0
Cadophora spp.	0	0	0	0
Cladosporium spp.	0	3	7	5
Exophila xenobiotica	0	0	0	1
Myrothecium roridum	0	0	0	1
Olpidium brassicae	0	2	3	1
Penicillium brevicompactum	0	0	1	0
Penicillium spp.	0	5	9	4
Petriella asymmetrica	0	3	5	0
Phoma spp.	0	0	0	0
Trichoderma viride	0	0	0	0
Bacteria and nematodes				
Agrobacterium rhizogenes	0	1	2	1
Agrobacterium tumefaciens	0	0	0	0
Clavibacter sp.	0	0	2	0
Erwinia spp.	11	9	4	0
Nitrospira spp.	11	11	12	9
Pseudomonas (universal)	12	12	12	10
Xanthomonas spp.	9	9	11	3
Pratylenchus spp.	0	1	3	0
Universal probes				
Bacteria	12	12	12	9
Fungi	5	11	12	9
Nematode	7	8	9	7
Oomycete	3	5	9	5

Recycling of irrigation water started in week 10.

Table 14. Overview of fungi, oomycetes, bacteria and nematodes detected by microarray at any time during 2013 in five different cropping systems

	On roots					In solution				
	RTW	pSSF	fSSF	UV	Heat	RTW	pSSF	fSSF	UV	Heat
Nursery	A	B	C	D	E	A	B	C	D	E
Pathogens										
Alternaria solani				✓			✓		✓	
Colletotrichum acutatum	✓	✓	✓	✓	✓		✓	✓	✓	
Colletotrichum coccodes		✓	✓	✓	✓		✓			✓
Fusarium oxysporum	✓	✓	✓	✓	✓	✓		✓	✓	
Fusarium redolens	✓	✓	✓	✓	✓	✓		✓	✓	
Fusarium solani		✓				✓	✓			
Phytophthora arecae			✓							
Phytophthora cryptogea		✓								
Phytophthora nicotianae							✓		✓	
Plectosphaerella cucumerina	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pyrenochaeta lycopersici	✓	✓	✓	✓	✓		✓			
Pythium aphanidermatum								✓		
Pythium diclinum	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pythium echinulatum							✓		✓	
*Pythium irregulare**	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pythium megalacanthum		✓	✓							

Table 14. Cont'd

	On roots					In solution				
	RTW	pSSF	fSSF	UV	Heat	RTW	pSSF	fSSF	UV	Heat
Nursery	A	B	C	D	E	A	B	C	D	E
Pythium myriotylum	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pythium paroencandrum	✓	✓	✓	✓	✓	✓	✓	✓		✓
Pythium torulosum		✓								
Rhizoctonia solani		✓		✓	✓		✓			
*Spongospora subterranea**	✓	✓	✓	✓	✓	✓	✓	✓		✓
Thielaviopsis basicola				✓						
Verticillium albo-atrum		✓	✓					✓		
Verticillium dahliae			✓							
Verticillium nigrescens	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Saprophytes										
Aspergillus flavus	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Aspergillus sydowii				✓	✓		✓	✓	✓	
Aspergillus terreus	✓									
Aspergillus ustus		✓								
Cadophora spp.		✓	✓							
Cephalosporium spp.			✓							
Cladosporium spp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table 14. Cont'd

	On roots					In solution				
	RTW	pSSF	fSSF	UV	Heat	RTW	pSSF	fSSF	UV	Heat
Nursery	A	B	C	D	E	A	B	C	D	E
Exophiala pisciphila		✓	✓		✓			✓		
Exophiala xenobiotica					✓			✓		✓
Myrothecium roridum		✓			✓					
Olpidium brassicae	✓	✓	✓	✓	✓	✓	✓			✓
Penicillium brevicompactum	✓	✓	✓	✓	✓	✓		✓		✓
Penicillium griseofulvum		✓	✓							
Pencillium variabile	✓	✓	✓			✓		✓		
Penicillium spp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Petriella asymmetrica	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Phoma sp.		✓								
Trichoderma viride		✓								
Trichoderma spp.									✓	
Bacteria										
Agrobacterium rhizogenes	✓	✓	✓	✓	✓	✓	✓			
Agrobacterium tumefaciens	✓	✓							✓	

Table 14. Cont'd

	On roots					In solution				
	RTW	pSSF	fSSF	UV	Heat	RTW	pSSF	fSSF	UV	Heat
Nursery	A	B	C	D	E	A	B	C	D	E
Bacillus amyloliquefaciens		✓	✓			✓	✓			
Bacillus subtilis		✓	✓			✓	✓	✓		
Clavibacter sp.		✓	✓	✓	✓		✓	✓		✓
Erwinia spp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Nitrospira spp. *(nitrite oxidising bacterium)*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pseudomonas universal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rhizobium sp.			✓							
Xanthomonas spp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Yersinia spp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Nematodes										
Pratylenchus spp.	✓	✓	✓	✓	✓	✓				
Number taxa detected										
Fungal & oomycete pathogens (31)	11	18	16	14	13	11	15	12	11	8
Fungal & oomycete saprophytes (29)	8	14	11	7	10	7	6	9	6	7
Bacteria (13)	7	10	10	7	7	9	9	8	6	6
Nematodes (3)	1	1	1	1	1	1	0	0	0	0
Total fungi and oomycetes (60)	19	32	27	21	23	18	21	21	17	15

*Specificity not confirmed; possible false positive results

Table 15. Microorganisms tested for by microarray and detected in all five rockwool crops

Fungal pathogens	Fungal saprophytes	Bacteria
Colletotrichum acutatum	*Aspergillus flavus*	*Agrobacterium rhizogenes*
Fusarium oxysporum	*Olpidium brassicae*	*Erwinia* spp.
Fusarium redolens	*Penicillium brevicompactum*	*Nitrospira* spp.
Plectosphaerella cucumerina	*Penicillium* spp.	*Pseudomonas universal*
Pyrenochaeta lycopersici	*Petriella asymmetrica*	*Xanthomonas* spp.
Pythium diclinum	*Yersinia* spp.	
*Pythium irregulare**		
Pythium myriotylum		
Pythium paroencandrum		
*Spongospora subterranea**		
Verticillium nigrescens		

*Specificity not confirmed; possible false positive results.

Table 16. Microorganisms tested for by microarray and not detected in any crop

Fungal pathogens	Fungal saprophytes	Bacteria
Armillaria mellea	*Alternaria* spp.	*Escherichia coli*
Didymella lycopersici	*Aspergillus terreus*	*Pseudomonas syringae*
Phytophthora cinnamomi	*Chaetomium cochlioides*	
Pythium arrhenomanes	*Chaetomium* spp.	
Pythium debaryum	*Doratomyces microsporus*	
Pythium oligandron	*Gigaspora rosea*	
Thielaviopsis basicola	*Gliocladium roseum*	
	Glomus intraradices	
	Penicillium chrysogenum	
	Rhizopus oryzae	
	Trichoderma harzianum	

Table 17. Comparison of rockwool microbial rhizosphere diversity (number of taxa) with different irrigation and water treatment systems (root data only)

Component		Run to waste	Recycled water			
			fSSF	pSSF	UV	Heat
Number of pathogenic fungal groups detected	Week 4	8	7	7	-	-
	Week 10	8	7	4	6 (wk 8)	6 (wk 8)
	Week 20	10	11	9	8 (wk 16)	(wk 16)
	Week 30	6	9	11	9 (wk 26)	(wk 26)
Number saprophytic fungal groups detected	Week 4	4	4	5	-	-
	Week 10	4	6	4	3	0
	Week 20	4	6	5	5	5
	Week 30	4	6	7	4	6
Number bacterial groups detected	Week 4	4	6	6	-	-
	Week 10	5	7	4	5	4
	Week 20	5	4	5	5	5
	Week 30	3	4	7	5	6
Total pathogenic fungal score[a]	Week 4	57	33	46	-	-
	Week 10	59	38	21	19 (wk 8)	11
	Week 20	33	41	51	34 (wk 16)	33
	Week 30	28	35	69	39 (wk 26)	52
Total saprophytic fungal score[a]	Week 4	16	25	32	-	-
	Week 10	23	53	49	6	0
	Week 20	16	34	48	27	16
	Week 30	11	14	42	10	31
Total bacterial score[a]	Week 4	39	51	43	-	-
	Week 10	44	53	36	47	43
	Week 20	34	31	42	43	43
	Week 30	32	26	43	40	47

[a] Sum of replicate slab scores for all microorganisms. Highest total taken where there are two primer sets used for a microorganism.

Table 18. Comparison of selected microorganisms in rockwool cube roots and slab solution before planting (✓ indicates presence)

Microorganisms	Nursery A – Norfolk (RTW)		Nursery B – Norfolk (part SSF)		Nursery C – Yorks (full SSF)	
	Root	Solution	Root	Solution	Root	Solution
Pathogenic fungi						
Colletotrichum coccodes					✓	
Fusarium oxysporum	✓		✓	✓	✓	✓
Fusarium redolens	✓		✓	✓	✓	
Pythium diclinum						
Pythium irregulare	✓		✓		✓	✓
Pythium megalacanthum						
Pythium myriotylum	✓					
Pythium paroencandrum	✓	✓	✓		✓	
Pythium torulosum						
Plectosphaerella cucumerina	✓		✓		✓	✓
Verticillium nigrescens	✓		✓		✓	✓
Saprophytic fungi						
Aspergillus flavus	✓		✓		✓	
Aspergillus ustus						
Cadophora spp.						
Cladosporium spp.	✓	✓	✓	✓	✓	✓
Myrothecium roridum						
Olpidium brassicae			✓		✓	
Penicillium brevicompactum	✓		✓		✓	
Penicillium spp.	✓		✓	✓	✓	
Petriella asymmetrica	✓	✓	✓	✓	✓	
Phoma spp.						
Trichoderma viride						
Bacteria and nematodes						
Agrobacterium rhizogenes			✓		✓	
Agrobacterium tumefaciens			✓			
Bacillus subtilis		✓				
Erwinia spp.	✓		✓		✓	
Nitrospira spp.	✓	✓	✓	✓	✓	✓

Table 18. Cont'd

Microorganisms	Nursery A – Norfolk (part RTW)		Nursery B – Norfolk (part SSF)		Nursery C – Yorks (full SSF)	
	Root	Solution	Root	Solution	Root	Solution
Pseudomonas (universal)	✓	✓	✓	✓	✓	✓
Xanthomonas spp.	✓	✓	✓		✓	
Pratylenchus spp.			✓			
Universal probes						
Bacteria	✓	✓	✓		✓	✓
Fungi	✓	✓	✓	✓	✓	✓
Nematode	✓			✓	✓	
Oomycete	✓		✓	✓	✓	✓

Following examination of the results in the preceding tables, the following observations were made:

Occurrence of specific microorganisms

Some microorganisms were detected on each or most sample occasions, usually at a relatively high abundance, and usually in all three of the crops monitored regularly (Table 19).

Table 19. Microorganisms commonly detected on most sample occasions and usually in all three sampled crops – 2013

Fungal and oomycete pathogens	Fungal saprophytes	Bacteria
Fusarium oxysporum	*Aspergillus flavus*	*(Agrobacterium rhizogenes)*
Fusarium redolens	*(Olpidium brassicae)*	*Nitrospira* spp.
Plectosphaerella cucumerina	*Penicillium* spp.	*Pseudomonas* spp.
Verticillium nigrescens	*Petriella asymmetrica*	*Xanthomonas* spp.
Pythium diclinum		
Pythium irregulare		
Pythium myriotylum		
Pythium paroencandrum		

( ) – common in one or two but not all three crops.

The frequent detection of *Fusarium oxysporum, Plectosphaerella cucumerina* and *Verticillium nigrescens* in the roots of rockwool crops confirm the findings of PC 281 where T-RFLP was used to monitor populations.

Olpidium brassicae is commonly found in the roots of hydroponic tomato crops. Although a pathogen on tobacco and a virus vector on lettuce, it is generally considered as a secondary opportunist, or at best a weak pathogen on tomato. Decomposed roots in hydroponic crops are often invaded by *Olpidium brassicae*. In this work it is therefore grouped with the saprophytes.

Some microorganisms were detected part way through the season and then tended to persist (Table 20).

Table 20. Microorganisms first detected on tomato roots partway through the season which subsequently tended to persist

Microorganism	Cropping system	First detection (week no)
Colletotrichum coccodes	pSSF	20
Colletotrichum coccodes	fSSF	36
Rhizoctonia solani	pSSF	10
Pythium myriotylum	fSSF	18
Spongospora subterranea	fSSF	16
Verticillium albo-atrum	fSSF	13

Conversely, some microorganisms were detected early (at one or more of the first three samplings) and not subsequently (Table 21).

Table 21. Microorganisms detected early in cropping in 2013, and not subsequently

Microorganism	Cropping system	First detection (week no)
Pythium megalacanthum	pSSF	2
Pythium torulosum	pSSF	2
Cadophora sp.	pSSF	2
Myrothecium roridum	pSSF	2
Trichoderma viride	pSSF	2
Pythium megalacanthum	fSSF	8
Pythium megalacanthum	pSSF	2

Some microorganisms were detected commonly (>3 times) at one site/in one cropping system only (Table 22).

Table 22. Microorganisms that were commonly detected on tomato roots at one site and rare or absent at the other two sites - 2013

Nursery A (RTW)	Nursery B (pSSF)	Nursery B (fSSF)
None	*Rhizoctonia solani* *Agrobacterium tumefaciens*	*Verticillium albo-atrum*

The common occurrence of *R. solani* (and also *C. coccodes* and *O. brassicae*) at site B may be associated with a soil processing operation (from sugar beet) adjacent to the glasshouse, possibly through wind-blown soil directly into the glasshouse or into an open lagoon where water was abstracted to irrigate the crop. These species occur commonly in cultivated soil.

Species richness

Neither of the two mycorrhizal fungi detected in PC 281 by T-RFLP (*Gigaspora rosea, Glomus intraradices*) was detected by the microarray It is possible that these were mis-identifications by T-RFLP. The primers used on the microarray provide a more specific identification than is possible by fragment length as used in T-RFLP.

Most of the commonly recognised antagonistic microorganisms were either not detected (*Gliocladium roseum, Trichoderma harzianum, Trichoderma* spp.) or rarely detected (*Bacillus amyloliquefaciens, Bacillus subtilis*). Only *Pseudomonas* (universal) was commonly detected (at a high level and in all cropping systems). The microarray does not provide information on which *Pseudomonas* species are present.

Some commonly recognised aggressive root pathogens were detected in one or two systems on one or two occasions: *Phytophthora cryptogea, Phytophthora nicotianae, Pythium aphanidermatum*.

The total number of fungal and oomycete pathogens and saprophytes was greater in the pSSF crop (42 in total), than the fSSF (36 in total) and RTW (26 in total) (Table 14).

Some *Pythium* spp. were detected commonly and some occasionally (Table 23).

Table 23. Frequency of detection of *Pythium* species in three tomato crops - 2013

Pythium species	Cropping system			Frequency of detection (proportion of sample occasions)		
	Nursery A (RTW)	Nursery B (pSSF)	Nursery C (fSSF)	Nursery A (RTW)	Nursery B (pSSF)	Nursery C (fSSF)
Common						
Pythium diclinum	✓	✓	✓	$^{9}/_{14}$	$^{16}/_{20}$	$^{9}/_{14}$
Pythium irregulare	✓	✓	✓	$^{14}/_{14}$	$^{17}/_{20}$	$^{14}/_{14}$
Pythium myriotylum	✓	✓	✓	$^{8}/_{14}$	$^{16}/_{20}$	$^{9}/_{14}$
Pythium paroencandrum	✓	✓	✓	$^{11}/_{14}$	$^{12}/_{20}$	$^{9}/_{14}$
Occasional						
Pythium aphanidermatum			✓	$^{0}/_{14}$	$^{0}/_{20}$	$^{1}/_{14}$
Pythium echinulatum		✓		$^{0}/_{14}$	$^{1}/_{20}$	$^{0}/_{14}$
Pythium megalacanthum		✓	✓	$^{0}/_{14}$	$^{1}/_{20}$	$^{1}/_{14}$
Pythium torulosum		✓		$^{0}/_{14}$	$^{1}/_{20}$	$^{0}/_{14}$

More microorganisms were detected on roots than in slab solution, for all cropping systems. Results for slab solutions are not given in this report as they are based on one UK sample, compared with four separate samples for the rhizosphere, and because they generally matched the rhizosphere though with fewer species.

Species diversity

Raw data from the microarray was first filtered removing any data below an intensity of 0.1 which was considered ambiguous. The Shannon Index (H'), a measure of species diversity, was then calculated for each of the arrays (Shannon, 1948). The mean species diversity was then plotted against week for each of the irrigations systems (Fig.1). During weeks 1-18 all three irrigation systems showed similar levels of diversity, however, from week 19 to 35 a clear difference in the levels of diversity was observed with a pattern of fSSF>pSSF>RTW on all but week 21 where the diversity of the fSSF system briefly drops below that of pSSF.

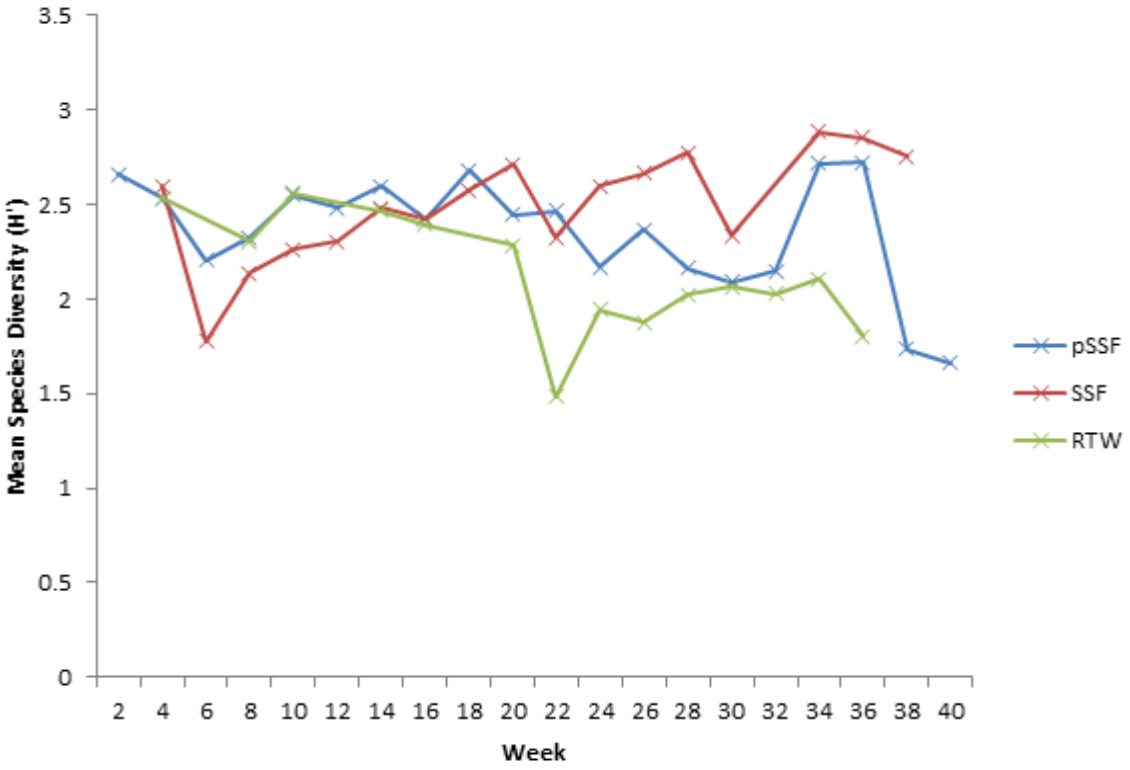

Fig.1 Mean species diversity (H') of rhizosphere associated microorganisms on tomato from three contrasting irrigation systems, pSSF, fSSF and RTW across a growing season. All plants were on Maxifort$_{TM}$ (De Ruiter) root stocks grown in rockwool during the 2012-2013 season.

The probes on the array, nematodes omitted, were also divided into their respective phylogenetic groupings of fungi, oomycetes and bacteria and analysed. An adjusted model Two-Way ANOVA was used to test the main effects of irrigation and week on species diversity (Table 24). Where a significant outcome was observed, a Tukey's Post hoc test was run to identify between which groups a significant difference was occurring.

Table 24. A summary of significant results from the Two-Way adjusted model ANOVA testing the mean differences between irrigation system (pSSF, fSSF and RTW) and week. For the ANOVA, probability (p) and effect size (n^2) are given and where possible the direction of variance, significance and mean difference is given for the post-hoc Tukey's test

Species diversity	Sample	Dependant variable	Statistical Summary				
					Post-hoc Tests		
			p	n^2	Direction	p	Mean difference (±)
Combined	Root	Irrigation	<0.001	0.220	pSSF>RTW	<0.001	0.288
					fSSF>RTW	<0.001	0.335
		Week	<0.001	0.258	2>4	0.015	0.523
					2>20	0.047	0.427
					4<12	0.015	0.525
					4<16	0.003	0.636
					4<18	0.036	0.489
					4<32	0.044	0.486
					12>20	0.048	0.426
					16>20	0.011	0.537
Fungal	Root	Irrigation	0.001	0.392	pSSF>RTW	0.007	0.372
					fSSF>RTW	<0.001	0.499
Oomycete	Root	Week	0.013	0.609	-	-	-

A significant difference in the mean diversities was found between the pSSF-RTW and fSSF-RTW systems for both the combined and fungal diversities from the root samples. The RTW system showed significantly less diversity across the year which can be seen in Fig. 2.

Significant differences in diversity were observed between week of the growing season on two occasions from the combined and oomycete diversities respectively. Post-hoc tests on the root-oomycete diversities were inconclusive.

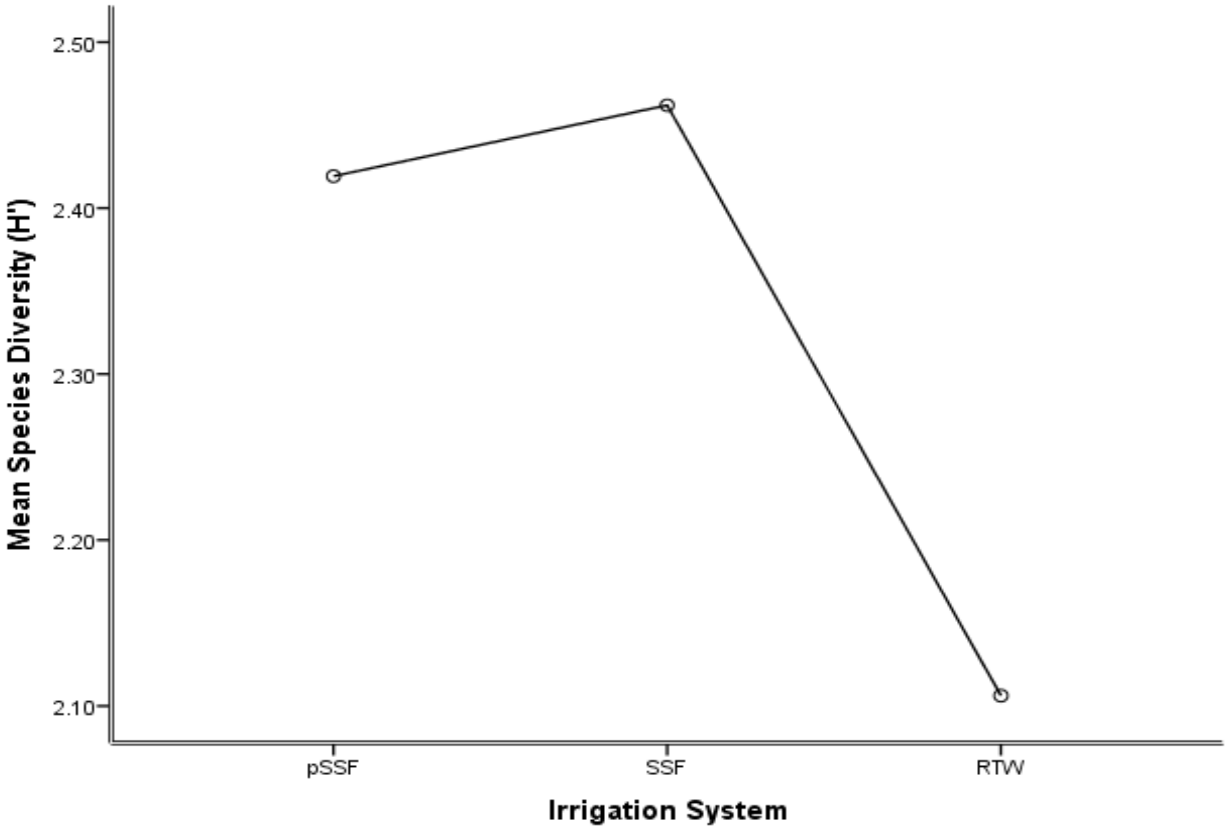

Fig. 2 The mean species diversity (H') of rhizosphere related microorganisms from rockwool tomatoes grown on contrasting irrigation systems (pSSF, SSF and RTW) over the 2012-2013 growing season.

How species changed over the growing season, independently of the irrigation system, was then analysed via Pearson's correlation co-efficient and Spearman's rank tests. Although general trends were observed for the phylogenetically split data, none were observed for the population as a whole. A summary of the findings can be seen in Table 25.

Table 25. A summary of the significant correlations (Pearson's correlation co-efficient) between species diversity and week across the 2012-2013 growing season. The table shows significance (p) and R identifying the strength (0-1) and direction of the relationship (±).

Species Diversity	Sample	p	R
Fungi	Root	0.012	-0.365
Oomycete	Root	<0.001	0.508
Bacteria	Root	0.003	-0.431

Three significant correlations were observed between species diversity and week. All, apart from oomycete, had a negative association with species diversity decreasing as the growing season progressed. Fungal and bacterial diversity, for example, showed two negative trends with R values of -0.365 and -0.43, respectively, whereas oomycete diversity had a moderate positive correlation of 0.508.

The observation of a decline in diversity at the roots over the growing season may be expected as levels of root exudate reduce with plant age reducing nutrients for the microbial population of the rhizosphere. Possibly there may also be some niche displacement as opportunist microorganisms colonise roots more strongly. Oomycete diversity increasing over the growing season may be a consequence of the hydroponic growing systems being a favourable environment for the oomycota.

Unexpected findings

The detection of some pathogens in one or more of the rockwool systems at more than isolated occurrences was unexpected to the author; notably *Colletotrichum acutatum* (normally a fruit pathogen); *Rhizoctonia solani, Pyrenochaeta lycopersici, Spongospora subterranea* (normally pathogens associated with soil cropping, although not unreported on soilless crops within Europe). The detection of *P. lycopersici* and *S. subterranea* may be due to cross hybridisation as both generally only showed low levels of hybridisation, often associated with the non-perfect base pairing of cross-hybridisation. This is supported by the probes specificity during validation. The RHSO probe, however, was shown to be species specific and its persistence between weeks 10-22 at site B may either be down to its rare incidence on soilless crops or contamination of the water source supplying the irrigation system from the neighbouring soil grown sugar beet crop. The presence of *Colletotrichum acutatum*, normally associated with anthracnose but scarcely reported on roots, was confirmed, by *C. acutatum* specific PCR

primers and DNA barcoding, for root and slab solution samples at sites A, B, C, D and E indicating that it may be more common on tomato roots than previously reported.

Treatment of recycled water

The crops grown on a recycling system with UV (nursery D) or heat treatment (nursery E) of water had very similar microbial populations to the other hydroponic crops.

Although there were some differences in the microbial populations on roots from UV and heat treated recycled water (Table 14), most of the microorganisms detected were present in both systems and the total number of species detected was similar.

Effect on crop growth

Tomato crops grown in rockwool slabs on rootstocks (Maxifort and Emperador) can carry a diverse population of potential fungal and bacterial pathogens on and/or in roots with no obvious detrimental effect on plant growth (i.e. no wilting or plant death). These results suggest that grafted plants on rockwool slabs can tolerate a certain level of one or more root pathogens without significant root damage. Possibly the high vigour of the rootstocks allows plants to produce sufficient new roots to compensate for those damaged by disease; and/or the diverse microbial population prevents increase in the pathogen inoculum to a level likely to cause severe root disease.

Root health and crop vigour

The appearance of roots in the sampled slabs and crop vigour at each sample occasion is shown in Table 26. It should be noted that these assessments were made by nursery staff and therefore comparisons between the nurseries should be treated with caution. At nursery A root health was good throughout the season. Crop vigour levels were very good until the middle of the season, when they began to fall gradually, becoming poor at the end of cropping. At nursery B both root health and crop vigour scores were generally good throughout the season, with root health dropping slightly at points and towards the end of the season and crop vigour remaining high throughout. At nursery C root health scores also begin high, but fall steadily through the season, resulting in very low scoring roots at the end. Crop vigour was awarded lower scores at nursery C than at nursery B throughout the season, also dropping to very low values by week 42. The weather early in 2013 was cold and dull and plants were reported as not overloaded with fruit. A high fruit load has previously been observed to result in a check to root growth and occurrence of some root death.

Table 26. Root health and crop vigour in the sampled areas of crop at time of assessment - 2013

Week number	Mean root health score (1-5)			Mean crop vigour score (1-5)		
	Nursery A (RTW)	Nursery B (pSSF)	Nursery C (fSSF)	Nursery A (RTW)	Nursery B (pSSF)	Nursery C (fSSF)
2	-	4	-	-	5	-
4	-	4	5	-	5	2
6	-	4	5	5	5	4
8	5	4	-	5	5	-
10	4	4.5	5	5	5	4
12	-	4	5	-	5	3.5
14	5	4.5	4.25	5	5	2.5
16	4	4.25	4.25	-	5	2
18	4	4.25	3.5	5	5	2
20	4	4.5	2	5	5	2
22	4	4	2	5	5	2
24	4	5	3.25	5	5	2
26	4	4.5	2.5	5	4	2
28	4	4.25	4	5	5	2
30	-	5	3.25	5	4	2
32	-	4.5	-	4	4	-
34	-	5	2.75	4	5	2
36	-	5	-	3	5	-
38	-	5	2.5	3	5	1
40	-	3.5	1.5	1	5	1
42	-	-	1	-	-	1
44	-	-	-	-	-	-

Root health was assessed in the 4 slabs at each sampling; 1 = very poor (nil or few white root tips); 5 = very good (no obvious rot or discolouration). Crop vigour was assessed by the host grower at 3 positions in the sampled row: 1 – very low vigour; 2 – low vigour. 3 – moderate vigour; 4 – strong vigour; 5 – very strong vigour. - = no data received.

Plant, root and stem base health towards end of cropping

An interim assessment of all sampled slabs was made in August, and a final assessment in mid-October as growers began to restrict crop watering in order to dry out the slabs.

At the interim assessment, all or nearly all plants remained alive and appeared healthy (Table 27). The few dead or missing plants were most likely due to stem breakage or Botrytis stem rot. The mean root health score was slightly less, and the proportion of slabs with a low health score was greater at site A (run-to-waste) than at sites B and C (water re-cycled). No vascular staining was found in any of the stem bases examined, not even at site C where plants on slabs where *V. albo-atrum* had been confirmed were examined.

Nursery A, run-to-waste: At the crop assessment on 30 August 2013, all the plants in monitored bags were alive, with no sign of plant wilting or yellowing. The mean root health score (3.3) was slightly less than that at nurseries B and C.

Nursery B, partial slow sand filter: At the crop assessment on 22 August, one plant was missing, one showed symptoms of root mat and one had obvious adventitious rooting on the lower portion of the stem.

Nursery C, full slow sand filter: There was no evidence that plant health was suffering due to root disease when the crop was assessed on 19 August. All stem bases were green and only one cube and two stems were missing, most probably due to physical breakage of the stem at crop layering. There were no symptoms of root mat disease. The lower stem on two plants (out of 144) showed adventitious rooting; both of these were in slabs where *V. albo-atrum* had been confirmed. The plants on 10 slabs (five where *V. albo-atrum* had been confirmed in roots and five where it was not found) were examined for vascular staining; none was found. Root health on 39 of 48 slabs was scored at index 4 or 5, which on the remaining slabs was index 3. There were no symptoms of *Fusarium* or *Verticillium* wilt in the top growth.

At the final assessment (Table 28) most stems remained in reasonable health at Nursery A and B, however a high percentage of plants at Nursery C were heavily stained and exhibited wilting, yellowing and missing stems. Nursery A had a similar level of missing stems as C, but Nursery B had relatively fewer missing stems in comparison. Plants in Nurseries A and B showed similar levels of vascular browning (0.2 – 0.3%), whereas Nursery C exhibited notably higher levels (6.8%). Adventitious rooting was not found at any of the sites. Root health at all 3 nurseries had deteriorated at the end of the season, though at Nursery A watering had already been stopped, which may have had some effect. Overall root health appeared best at Nursery B (pSSF) and worst at Nursery C (fSSF). No root mat disease symptoms were recorded at any nursery.

A single assessment was made on 30 September at sites D and E (water re-cycled, with UV and heat treatment respectively). As at sites A-C, all or nearly all plants were alive and appeared healthy. Vascular staining was obvious in 3/63 stems at site D and in 4/62 stems at site E (Table 29). Five of these seven plants had healthy plant tops. Microarray tests on stained stem base vascular tissue indicated occurrence of *Alternaria solani, Plectosphaerella cucumerina, Pyrenochaeta lycopersici, Phytophthora cryptogea, Verticillium nigrescens* and *Xanthomonas* spp. The crop at site D (UV) had a greater occurrence of adventitious rooting at the stem base and a lower root health score than that at site E (heat treatment).

The growers were satisfied with crop performance and yield in all five crops.

Table 27. Summary of interim (August 2013) root and stem base assessments on sampled plants, nurseries A-C

	Nursery A (RTW)	Nursery B (pSSF)	Nursery C (fSSF)
Date assessed	30 Aug	22 Aug	19 Aug
Number slabs assessed	92	65	52
Number stem bases assessed	366	260	312
Number stem bases alive (green)	366	259	307
Number stem bases yellowing	0	0	0
Number stem bases dead/missing	0	1	5
Mean root health score (0-5)	3.3	3.8	3.8
Number slabs with root health ≤3	51	11	11
Number stems with adventitious roots	0	1	2
Number stem bases with vascular stain	0/48	0/40	0/60[a]
Number cubes with root mat	0	1	0

[a] 5 slabs where *V. albo-atrum* had been confirmed and five where this fungus was not found.

Table 28. Summary of final root and stem base assessment on sampled plants, nurseries A-C, October 2013

	Nursery A (RTW)	Nursery B (pSSF)	Nursery C (fSSF)
Date of final assessment	25 Oct	17 Oct	30 Oct
Number slabs assessed	104	73	86
Number stems assessed	416	292	516
Stem health			
% stems alive (green)	98.1	100	89.2
% stems yellowing	0	0	2.1
% stem dead/missing	1.7	0.3	1.9
% stem with vascular browning	0.2	0.3	6.8
Mean vascular brown score (0-3) of affected stems	1	1	1.8
% stems with adventitious rooting	0	0	0
Root appearance			
Mean root health score (1-5)	2.8	3.5	2.4
% plants with score ≤3	47.6	46	98.8
% plants with root mat	0	0	0
Pathogens confirmed* (conventional tests)			
Botrytis cinerea			✓
Colletotrichum coccodes		✓	
Fusarium spp.	✓	✓	✓
Pythium spp.	✓	✓	✓
Pathogens confirmed* (microarray)			
Agrobacterium tumefaciens			✓
Colletotrichum coccodes		✓	✓
Erwinia spp.	✓		✓
Fusarium oxysporum	✓		✓
Fusarium redolens		✓	✓
Fusarium solani		✓	
Pythium diclinum		✓	✓
Pythium irregulare	✓	✓	✓

Table 28. Cont'd

	Nursery A (RTW)	Nursery B (pSSF)	Nursery C (fSSF)
Pythium myriotylum		✓	✓
Pythium paroencandrum	✓		
Plectosphaerella cucumerina	✓	✓	✓
Pyrenochaeta lycopersici		✓	✓
Spongospora subterranea		✓	
Verticillium albo-atrum			✓
Verticillium nigrescens	✓	✓	✓
Xanthomonas spp.		✓	✓

* On roots and stems sampled at the final assessment.

Table 29. Summary of final root and stem base assessment on sampled plants, nurseries D and E – September 2013

	Nursery D (UV)	Nursery E (Heat)
Date assessed	20 Sep	20 Sep
Number slabs assessed	16	16
Number stem bases assessed	64	64
<u>Stem health</u>		
Number stem bases alive (green)	63	62
Number stem bases yellowing	0	2
Number stem bases dead/missing	1	0
Number stems with adventitious roots	21/63	0/62
Number stem bases with vascular stain	3/63	4/62[a]
<u>Root appearance</u>		
Mean root health score (1-5)	3.2	3.5
Number slabs with root health ≤3	3	1
Number cubes with root mat	0	0

[a] Two of the 4 plants with vascular staining had yellowing stems and girdling Botrytis lesions on the upper stem, resulting in plant wilting.

Objective 2b – Investigate tomato root diseases by microarray and conventional tests

Introduction

In addition to the routine monitoring (Objective 2a), a second approach was taken to determine if rhizosphere microbial populations could be used to identify crops at risk from serious root disease. The microbial populations on roots of plants affected and unaffected by serious root or vascular disease in the same crop were compared.

Materials and methods

Requests to growers to notify ADAS of crops affected by a suspected root disease were made through HDC and TGA communications. Crops inspected during ADAS consultancy work were also examined. Samples collected were tested by both microarray and conventional tests.

Results and discussion

No reports of serious root disease problems in rockwool crops were received in 2013. A limited number of samples with root disease problems were collected during consultancy work, primarily from crops growing in soil or coir.

Details of the commercial crops with suspect root disease problems examined in the project are given in Table 30.

A range of potential pathogens were identified from the microarray and conventional test results (Tables 31-32). The microarray consistently identified a greater number of potential pathogens than the conventional tests. The potential pathogens were generally detected in root samples from both ‘good’ and ‘poor’ plants taken from the same crop. This indicates a higher incidence of root disease was present than was apparent from top growth, the disease having caused greater damage to root function in some plants than others. There was no clearly identified single cause of disease in any of the samples.

No *Agrobacterium rhizogenes* was detected in cube and slab samples with root mat symptoms. These roots were infected with *Pythium* spp. and *Fusarium oxysporum*, although not all at detectably greater levels than in plants from the same crops without root mat symptoms.

Opportunity was also taken to determine if an addition of a liquid fertiliser affected root zone microbial populations. At nursery B, the effect of application of Biome Plus, a liquid fertiliser

containing plant growth promoting bacteria, including *Bacillus amyloliquefaciens* strain FXB42 was applied by the grower through the irrigation lines on 31 July. Roots were sampled on 20 August 2013. There was no obvious difference in the microbial populations on roots of treated and untreated plants (Table 33). No *Bacillus* species were detected.

Table 30. Comparison of predominant potential pathogens and antagonists (shaded rows) detected on tomato roots of paired samples ('good' and 'poor') by microarray and conventional tests

Sample and symptom	Microorganism	Microarray relative abundance		Conventional test relative abundance			
		Good	Poor	Good		Poor	
				Isolation	Float	Isolation	Float
BX13/35	*C. coccodes*	8/18	5/18				
Soil - wet area with yellowing leaves	*F. oxysporum*	10/18	8/18	1/12			
	Fusarium sp.	-	-			10/12	
	P. cryptogea	9/9	9/9	12/12			
	Pythium spp.	20/27	7/20		✓	12/12	
	Plectosphaerella	14/18	11/18				
	Pyrenochaeta	18/18	15/18				
	Thielaviopsis	1/9	0/9				
	V. nigrescens	7/9	4/9				
	Penicillium spp.	0/9	1/9				
BX13/36	*F. oxysporum*	0/18	4/18				
Coir slab – slab root mat	*Fusarium* sp.	-	-	4/12		4/12	
	Pythium spp.	11/27	3/27	8/12		4/12	✓
	Plectosphaerella	3/18	6/18				
	V. nigrescens	0/9	4/9				
	Agrobacterium rhizogenes	1/9	0/9				
	Penicillium spp.	0/9	2/9				
BX13/37	*C. coccodes*	3/18	3/18				
Soil – thin head, wilting	*Phytophthora* sp.	0/9	1/9				
	Pythium spp.	4/9	1/9	12/12		0/12	✓
	Plectosphaerella	8/18	2/18				
	Pyrenochaeta	13/18	15/18				
	V. nigrescens	4/9	1/9				
	Fusarium sp.	-	-	0/12		6/12	

Table 30. Cont'd

Sample and symptom	Microorganism	Microarray relative abundance		Conventional test relative abundance			
		Good	Poor	Good		Poor	
				Isolation	Float	Isolation	Float
	Penicillium sp.	0/9	1/9				
	Trichoderma sp.			5/8		0/8	
BX13/38	*C. coccodes*	0/18	1/18				
Coir slab – suspect FCRR	*F. oxysporum*	4/18	1/18				
	F. solani	2/9	0/9				
	Fusarium sp.	-	-	1/12		4/12	
	Pythium sp.	1/27	4/27	0/12		4/12	✓
	Plectosphaerella	13/18	4/18				
	V. nigrescens	5/9	2/9				
	Penicillium spp.	3/9	1/9				
	Trichoderma spp.	2/9	1/9	1/12		4/12	
	Bacillus subtilis	0/9	4/9				
BX13/39	*F. oxysporum*	0/18	5/18				
Coir slab – Cube root mat	*Fusarium* sp.	-	-	1/12		1/12	
	Pythium spp.	12/36	8/36	4/12		1/12	✓
	Plectosphaerella	5/18	4/18				
	Pyrenochaeta	1/18	6/18				
	V. nigrescens	2/9	3/9				
	Agrobacterium rhizogenes	1/9	0/9				
	Penicillium spp.	4/18	4/18				
	Trichoderma spp.	1/9	0/9	4/12		0/12	

Unshaded rows indicate potential pathogens. Shaded rows indicate saprophytes.

Blanks under conventional tests indicate the organism was not recovered.

Table 31. Summary of probable causes of poor growth in paired samples – June 2013

Sample reference	Substrate and symptoms	Probable causal pathogens
BX13/35	Soil crop; wet area with yellowing lower leaves	*Fusarium oxysporum*
		Phytophthora cryptogea
		Pythium spp.
		Pyrenochaeta lycopersici
		Verticillium nigrescens
BX13/36	Coir slab crop; slab root mat	*Fusarium oxysporum*
		Pythium spp.
BX13/37	Soil crop; thin heads, wilting plants	*Colletotrichum coccodes*
		Pyrenochaeta lycopersici
BX13/38	Coir slab crop; cube root mat	*Fusarium oxysporum*
		Pythium spp.
		Pyrenochaeta lycopersici
BX13/39	Coir slab crop; suspect FCRR	*Pythium* spp.

Table 32. Root samples from crops with rot, discolouration or other symptom of a suspect root disease problem – 2013

Sample reference	Date received	Symptom	Main pathogens detected	
			Conventional test	Microarray
1. BX13/09 (Kent, Dometica own roots)	11 Feb	Soft, dark brown roots at RW slab top	Bacteria + *Fusarium*	**Bacteria** + *Fusarium* + others
2. BX13/15 (Norfolk, Roterno on Maxifort)	5 March	Dark brown roots, RW lab base	*Fusarium oxysporum* *Trichoderma*	
3. BX13/16 W. Sussex, Mecano on Maxifort	6 March	Pale brown roots, RW slab base	*Fusarium*	
4. BX13/35 (IoW, Lierne on Maxifort)	4 June	Wet area (yellowing leaves) Soil crop	*Fusarium* *Pythium*	***C. coccodes*** *F. oxysporum* ***P. cryptogea*** ***Pyrenochaeta*** *Pythium* spp.
5. BX13/36 (IoW, Angele on Maxifort)	4 June	Root mat on coir slabs	*Fusarium* *Pythium*	*F. oxysporum* *Pythium* spp.
6. BX13/37 (IoW, Capri on Beaufort)	4 June	Thin head, wilting plants, soil crop	*Fusarium* *Pythium*	***C. coccodes*** ***Pyrenochaeta***
7. BX13/38 (IoW, Arlinta on own roots)	4 June	Suspect Fusarium crown and root rot on coir slab	*Fusarium* *Pythium*	*C. coccodes* *F. oxysporum* *Pythium*
8. BX13/39 (IoW, Angelle on Beaufort)	4 June	Root mat on coir cube	*Fusarium* *Pythium*	*F. oxysporum* *Pyrenochaeta* *Pythium*

Bold – suspect main causal microorganisms, judged on symptoms and frequency of occurrence.

Table 33. Effect of Biomax-Plus treatment applied through irrigation lines on microbial populations associated with roots – nursery B, week 31

Microorganism	Untreated	Biomex-Plus* treated
Fusarium oxysporum	✓	
Fusarium redolens		✓
Pythium diclinum	✓	✓
Pythium irregulare	✓	
Pythium myriotylum	✓	✓
Pythium paroencandrum	✓	
Plectosphaerella cucumerina	✓	✓
Pyrenochaeta lycopersici	✓	
Verticillium nigrescens	✓	✓
Aspergillus flavus	✓	
Cladosporium spp.	✓	✓
Olpidium brassicae	✓	✓
Penicillium variabile	✓	✓
Penicillium spp.	✓	✓
Petriella asymmetrica	✓	
Erwinia sp.	✓	
Nitrospira spp.	✓	✓
Pseudomonas universal	✓	✓
Xanthomonas spp.	✓	✓
Pratylenchus sp.	✓	✓
Bacteria universal	✓	✓
Fungal universal	✓	✓
Nematode universal	✓	
Oomycete universal	✓	✓

* Biomex Plus is described as a liquid fertilizer containing plant growth promoting rhizobacteria; the bacterium *Bacillus amyloliquefaciens* strain FZB42 is listed as a component.

Objective 3 – Examine microorganism population changes with reference to root zone physical environment

Introduction

The growth and pathogenicity of rhizosphere microorganisms will be influenced by the root zone physical environment, including slab moisture content, solution pH, temperature and conductivity: for example, *Pythium* and *Phytophthora* spp. are favoured by high moisture content; infection by *Pythium* spp. is favoured by high conductivity; *Pythium aphanidermatum* has a high (35-40 °C) optimal growth temperature. The objective in this project was to examine data on some root zone physical factors recorded by growers at the host nurseries (Objective 2 – routine root monitoring) alongside microarray results and determine if there were any marked changes that appeared to be correlated.

Materials and methods

Host nurseries were requested to supply data on root zone pH, conductivity, nutrition and temperature. Rhizosphere microorganism populations were monitored as described in Objective 2. Data were examined to record if there were any time points when root zone physical factors were outside accepted tolerance levels; or there was a large change (≥20%) between mean values on successive days.

Results and discussion

Root zone physical factors

Data on pH, conductivity and temperature are summarised in Table 34. On no occasions did the host nursery managers consider that root zone physical factors were outside tolerance levels (Appendix 3).

Table 34. Detailed pH, EC and temperature values from Nurseries B and C (no detailed data supplied by Nursery A).

Week no.	Nursery B			Nursery C		
	pH	EC	Temp	pH	EC	Temp
4	6.8	4.7	17.23	6.3	10.3	17.1
6	6.6	4.4	16.76	6.43	5.53	17.1
8	6.9	4.2	17.87	-	-	17.6
10	6.0	3.8	19.04	6.6	3.2	17.9
12	5.9	3.9	19.14	-	4.0	17.7
14	5.7	3.5	18.45	-	-	17.8
16	5.8	3.2	20.22	5.9	3.4	18.5
18	6.3	3.2	20.07	6.18	4.38	18.7
20	5.8	3.3	20.44	-	-	19.0
22	5.9	3.2	20.20	6.49	4.8	18.7
24	6.7	3.9	19.79	-	-	19.2
26	5.9	3.8	20.50	6.23	4.11	19.0
28	6.1	3.9	20.66	6.21	4.57	19.6
30	5.9	3.4	22.04	6.81	4.32	20.1
32	6.4	3.5	23.46	-	-	20.4
34	6.8	3.3	22.73	-	-	19.4
35	7.8	3.8	21.31	-	-	19*.3
38	7.6	3.8	21.83	5.36	3.43	18.3
40	7.6	4.5	17.73	6.52	5.45	19.2
42	6.8	4.7	20.10	-	-	18.9

- no data available.

Rhizosphere microbial populations

It was striking that most microorganisms recorded at a moderate or high relative abundance on at least one occasion were detected on all sample occasions (Tables 8-10); and microorganisms recorded at a low relative abundance generally occurred more sporadically; i.e. the microbial population diversity at each site was largely stable.

The effect of time on total species diversity was examined and discussed in detail in Objective 2a. The main points were: a) species diversity dips in all three nurseries at Week 6; b) differences between certain weeks are summarised in Table 24.

There was no obvious explanation for the dip in species diversity in week 6; this was well before peak fruit load when root dieback is noted to occur. It was also before the crops at nurseries B and C were switched from run-to-waste to re-circulation (Week 10). There was no large change in any of the recorded root zone physical factors around week 6. Possibly there was some difference in the DNA extraction and testing of these three samples that resulted in a lower DNA recovery. It is suggested that the effect of root zone physical factors on rhizosphere microbial populations would be better studied through replicated experiments, with one factor at a time deliberately altered for a defined period.

Objective 4 – Develop and validate LAMP assay

Introduction

In-house rapid point-of-care detection of potential pathogens is an emerging element of the commercial tomato growers integrated pest management scheme. The ability to accurately detect pathogenic microorganisms allows the grower to take early preventative steps and thus minimise disease dissemination. A new technology known as LAMP, loop-mediated isothermal amplification, allows the rapid, sensitive and specific detection of causal agents of disease. LAMP tests have a greater level of specificity and sensitivity than the microarray for detection of microorganisms. Also, unlike the microarray, it is possible to do tests on-site using portable equipment. LAMP utilises a set of six primers to initiate isothermal amplification. The strand displacement synthesis activity of the polymerase and use of six primers results in a highly specific and rapid amplification with the ability to discriminate between single nucleotide polymorphisms. In an end-to-end process that can be achieved in under an hour including nucleic acid extraction, with relatively little *a priori* knowledge, LAMP provides a portable, fast alternative to disease monitoring. The objective of this work was to develop LAMP assays for some important tomato pathogens.

Materials and methods

Primer design

During progress meetings potential targets were discussed with growers. These were *Colletotrichum coccodes, Verticillium albo-atrum, Fusarium oxysporum, Thielaviopsis basicola, Pyrenochaeta lycopersici, Botrytis cinerea* and viral diseases such as pepino mosaic virus. Diagnostic Lamp tests for pepino mosaic virus are already published, so we concentrated on fungal diseases of tomato. Potential gene sequences that could be used as marker genes from

which to design primers for detection of specific organisms were identified for the pathogens. These were mainly the intergenic sequences of the rDNA operon, but also specific sequences such as a cellulose and a tomatinase gene for *F. oxysporum*, and a sequence characterised amplified region (SCAR) marker for *V. albo-atrum.* *In silico* techniques were then used to identify regions of heterogeneity using software platform MEGA 5.1. From the identified regions LAMP primers were designed using available primer design software such as PrimerExplorer V4 (Eiken chemical co.)/LAMP Designer (Premiere Biosoft). The primers were ordered from Sigma-Aldrich®.

Validation

Primers were then validated using reference cultures and DNA provided by FERA, ADAS and The University of Nottingham. All cultures and DNA were identified via classical morphological techniques and sequencing of their ITS region, a distinguished DNA barcoding region. A list of all DNA samples can be found in Table 2. All assays were run at 63°C for 45 minutes. Primers were initially self-validated and if successful run against a number of closely related and common organisms present in the tomato rhizosphere. If a positive result was observed anneal analysis was then performed. Anneal analysis allowed discrimination of any amplified products based on their relative GC content, and can subsequently be used as an in-built validation test in LAMP assays.

Results and discussion

A number of different primer sets were designed and tested for the detection of *P. lycopersici, V. albo-atrum*, *B. cinerea, C. coccodes, T. basicola* and *F. oxysporum*. Of these, three gave consistent amplification from the target organisms and no amplification from other organisms. These were the *B. cinerea* primers (designed based on the rRNA IGS sequences), the *C. coccodes* COLI primers (also designed based on the rRNA IGS sequences), and the *F. oxysporum* FOXYC primers (designed based on a cellulose gene). Typical amplification results for the FOXYC primers are shown in Table 35, where the amplification time is the time taken for a positive amplification, and the ‘Anneal’ is the temperature at which the target DNA anneals, which can be used as a validation assay for a positive result.

Table 35. A summary of the results from the FOXYC primer sets including their point of amplification (in minutes) and their subsequent anneal temperature.

Primer set	Organism	Amplification (mins)	Anneal (°C)
FOXYC	*Fusarium oxysporum (101)*	13.45	92.23
	Verticillium spp.	-	-
	Fusarium oxysporum (150)	13.15	92.35
	Fusarium graminearum	-	-
	Penicillium ochrochloron	-	-
	Botrytis cinerea	-	-
	Colletotrichum coccodes	-	-
	Aspergillus niger	-	-
	Epicoccum nigrum	-	-
	Fusarium acuminatum	-	-
	Fusarium solani	-	-
	Rhizoctonia solani	-	-
	Penicillium expansum	-	-
	Pyrenochaeta lycopersici	-	-
	Thielaviopsis basicola	-	-

All other sets of primers tested gave unsatisfactory results, either amplifying from a broad range of organisms along with the target organism (suggesting a lack of specificity with the primers) or failing to amplify from any organisms including the target (suggesting that the primer design software had failed to identify appropriate primers). For these other organisms, it is likely that target genes other than the rRNA need to be identified through further searches of sequence databases, and primers designed and tested based on these. However, three good robust tests have been developed for fungal pathogens of tomato.

Conclusions

Microarray validation

1. A microarray, for the detection of tomato rhizosphere microorganisms, developed in a separate project, was validated in this project using DNA from 75 species and 35 genera. Tests showed:

   a) Twenty eight of 60 fungal and oomycete species-specific probes were self-validated. Fifteen showed no or low level cross-hybridisation*: Alternaria solani, Aspergillus flavus, Aspergillus terreus, Chaetomium cochlioides, Colletotrichum coccodes* (1), *Exophiala pisciphila, Fusarium solani* (2), *Penicillium chrysogenum, Plectosphaerella cucumerina* (1), *Plectosphaerella cucumerina* (2), *Rhizoctonia solani, Thielaviopsis basicola* (1), *Thielaviopsis basicola* (2), *Trichoderma harzianum and Verticillium dahlia*e. Seventeen fungal and oomycete species-specific probes could not be tested for self-validation due to lack of suitable DNA

   b) Three out of eight fungal and oomycete genera-specific probes were self-validated and showed no cross-hybridisation. These were: *Alternaria* spp., *Phoma* spp. and *Trichoderma* spp.

   c) Eleven bacterial species or genera-specific probes were self-validated. Only two, *Escherichia coli* and *Ralstonia solanacearum* showed no cross hybridisation.

2. Levels of cross-hybridisation were shown to be reduced when samples consisted of community DNA, as used elsewhere in this project.

Tomato root microorganism monitoring

1. Tomato roots taken from five commercial crops grown on rootstocks on rockwool slabs were shown to have a diverse microbial population from soon after planting comprising at least 19-32 fungal and oomycete taxa and 7-10 bacterial taxa.

2. The diversity of the combined microbial populations was relatively stable throughout the year. However, fungal and bacterial species diversity decreased significantly as the growing season progressed, whereas oomycete species diversity increased. Overall, there were more similarities than differences between the five crops.

3. The number of fungal and oomycete taxa present on roots of Maxifort rootstock was greatest in a crop grown with the nutrient solution part recycled through a slow sand filter (32 taxa), and least in a crop grown with run-to-waste irrigation (19 taxa).

4. Tomato plants grown on Maxifort rootstock had a large number of potential fungal and oomycete pathogens commonly associated with their roots and yet showed no obvious adverse effect on crop growth. These include *Fusarium oxysporum*, various *Pythium* species and *Verticillium albo-atrum*.

5. Some microorganisms commonly occurred at a high frequency and abundance on tomato roots in rockwool cropping systems, notably: *Fusarium oxysporum, Plectosphaerella cucumerina, Verticillium nigrescens, Penicillium* spp., *Nitrospira* spp., *Pseudomonas* spp. and *Xanthomonas* spp.

6. Some *Pythium* species (*P. diclinum, P. irregulare, P. myriotylum, P. paroencandrum)* were more common in rockwool tomato crops than others (*P. aphanidermatum, P. echinulatum, P. megalacanthum, P. torulosum*).

7. Some microorganisms did not occur on roots until many weeks after planting, but tended to persist once they did occur. This may indicate either introduction to the crop part way through the season and/or alteration of the crop (e.g. root death) that allows their development. Such microorganisms include *Colletotrichum coccodes, Rhizoctonia solani* and *Verticillium albo-atrum.*

8. Some microorganisms were commonly detected at one site/in one cropping system only. This may relate to differences in the irrigation system and/or site differences. For example, *Verticillium albo-atrum* was only commonly detected in the crop with recycling all water through a slow sand filter; *Colletotrichum coccodes* and *Rhizoctonia solani* were only commonly found in the crop with part of the waste irrigation water recycled through a slow sand filter.

9. The finding of *Colletotrichum acutatum* to be commonly present on tomato roots was unexpected, subsequent DNA barcoding indicated this is a true identification. The finding of *Pyrenochaeta lycopersici* on crops in rockwool was also somewhat unexpected, and may indicate the fungus is common in many soils and is introduced into rockwool crop through windblown dust. Wherever tomatoes are grown in soil in the UK, brown and corky root rot usually occurs.

Use of microarray to detect root pathogens

10. Use of the tomato rhizosphere microarray indicated a wider range of potential pathogens associated with a root disease problem than standard conventional laboratory tests on roots.

11. The microarray has some advantages over conventional tests including rapid identification of slow growing fungi (e.g. *Pyrenochaeta lycopersici, Verticillium albo-atrum*), rapid identification to species level and discrimination of closely related species (e.g. species of *Pythium* and *Phytophthora*) normally requiring a high level of taxonomic expertise for correct identification.

LAMP assays

12. Lamp diagnostic assays have been developed and validated in this project for three fungal diseases of tomato, *Botrytis cinerea, Colletotrichum coccodes* and *Fusarium oxysporum.* These tests have been shown to give amplification of target DNA within 20 minutes using the real-time Lamp Genie II machine developed by Optigene UK, and there is now potential for these assays to be developed (along with Lamp assays that have already been developed by others such as for *Pepino mosaic virus*) into commercial test kits that could be used by growers in glasshouses. Other commercial diagnostic test kits based on Lamp are currently being developed by Optigene for users such as UK Plant Health Inspectors.

Technology transfer

Project meetings

Initiation meeting, Sutton Bonington, 18 January 2013

Review meeting, Sutton Bonington, 19 April 2013

Progress meeting/demonstration, Cornerways Nursery, 6 June 2013

Progress meeting, Sutton Bonington, 2 August 2013

Review meeting, Sutton Bonington, 4 November 2013

Presentations

New technologies for disease control. HDC/TGA Tomato Conference, Coventry, 26 September 2013 (Tim O'Neill)

Publications

O'Neill TM, Deery S, Scott G & Dickinson M. Monitoring tomato rhizosphere microorganisms (2014). Chemical and non-chemical Soil and Substrate Disinfestation Symposium, Italy, July 2014 (paper submitted for *Acta Horticulturae*).

O'Neill TM & Scott G (2013). Microbial secrets of roots laid bare. *HDC News* **199**, 16-17.

References

Shannon, C. E. (1948) A mathematical theory of communication. *The Bell System Technical Journal*, **27**, 379–423 and 623–656

Appendix 1 Crop diaries (routine monitoring)

Nursery A (West Sussex)		Nursery B (Norfolk)		Nursery C (Yorkshire)	
21 Jan	Sample 1 (from cubes)	8 Jan	Sample 1 (from cubes)	24 Jan	Sample 1 (from cubes)
24 Jan	Slab contact	24 Jan	Sample 2	25 Jan	Slab contact
21 Feb	Sample 2	25 Jan	Slab contact	5 Feb	Sample 2
6 Mar	Sample 3 (ADAS)	6 Feb	Recirculation starts	18 Feb	Sample 3 (ADAS)
9 Apr	Sample 4	5 Feb	Sample 3	4 Mar	Recirculation starts
16 Apr	Sample 5	19 Feb	Sample 4	5 Mar	Sample 4
17 May	Sample 6	5 Mar	Sample 5 (ADAS)	26 Mar	Sample 5
12 Jun	Sample 7	19 Mar	Sample 6	11 Apr	Sample 6
26 Jun	Sample 8	2 Apr	Sample 7	24 Apr	Sample 7
10 July	Sample 9	16 Apr	Sample 8	7 May	Sample 8
22 July	Sample 10	30 Apr	Sample 9	22 May	Sample 9
29 July	Sample 11				
12 Aug	Sample 12	14 May	Sample 10	6 Jun	Sample 10
27 Aug	Sample 13	28 May	Sample 11	24 Jun	Sample 11
30 Aug	Disease assessment	11 Jun	Sample 12	11 July	Sample 12
16 Sep	Sample 14	25 Jun	Sample 13	24 July	Sample 13
25 Oct	Final assessment	9 July	Sample 14	19 Aug	Sample 14 (ADAS)
		23 July	Sample 15	19 Aug	Disease assessment
		2 Aug	Sample 16	3 Sep	Sample 15
		21 Aug	Disease assessment	18 Sep	Sample 16
		20 Aug	Sample 17	31 Oct	Final assessment
		3 Sep	Sample 18		
		17 Sep	Sample 19		
		8 Oct	Sample 20		
		17 Oct	Final assessment		

Appendix 2 Crop diaries (periodic monitoring)

	Nursery D (Yorks - UV)	UV light		Nursery E (Yorks - Heat)	Pasteuriser
8 Jan	Plants arrive	-	8 Jan	Plants arrive	
18 Feb	Sample 1	-	18 Feb	Sample 1	
25 Feb	Start recycling	-	25 Feb	Start recycling	
22 Apr	Sample 2	On	22 Apr	Sample 2	Off
1 July	Sample 3	Off	1 July	Sample 3	On
20 Sep	Sample 4	On	20 Sep	Sample 4	On
20 Sep	Disease assessment		20 Sep	Disease assessment	

Appendix 3 pH, EC and nutrition (routine monitoring)

Week Number	Slab solution in acceptable (✓) or unacceptable (X) range								
	Nursery A			Nursery B			Nursery C		
	pH	EC	Nutrition	pH	EC	Nutrition	pH	EC	Nutrition
1									
2				✓	✓	✓			
3									
4				✓	✓	✓	✓	✓	✓
5									
6	✓	✓	✓	✓	✓	✓	✓	✓	✓
7									
8	✓	✓	✓	✓	✓	✓	✓	✓	✓
9									
10	✓	✓	✓	✓	✓	✓	✓	✓	✓
11				✓	✓	✓			
12				✓	✓	✓			
13							✓	✓	✓
14				✓	✓	✓			
15	✓	✓	✓				✓	✓	✓
16	✓	✓	✓	✓	✓	✓			
17							✓	✓	✓
18				✓	✓	✓			
19	✓	✓	✓				✓	✓	✓
20	✓	✓	✓	✓	✓	✓			
21							✓	✓	✓
22	✓	✓	✓	✓	✓	✓			
23							✓	✓	✓
24	✓	✓	✓	✓	✓	✓			
25									
26	✓	✓	✓	✓	✓	✓	✓	✓	✓
27									
28	✓	✓	✓	–	✓	✓	✓	✓	✓
29									
30	✓	✓	✓	✓	✓	✓	✓	✓	✓
31	✓	✓	✓						
32				✓	–	✓			
33	✓	✓	✓						
34				✓	✓	✓			
35	✓	✓	✓				✓	✓	✓
36				✓	✓	✓			
37									
38	✓	✓	✓	✓	✓	✓	✓	✓	✓
39									
40							✓	✓	✓
41	✓	✓	✓	✓	✓	✓			
42							✓	✓	✓

Appendix 4 Photographs

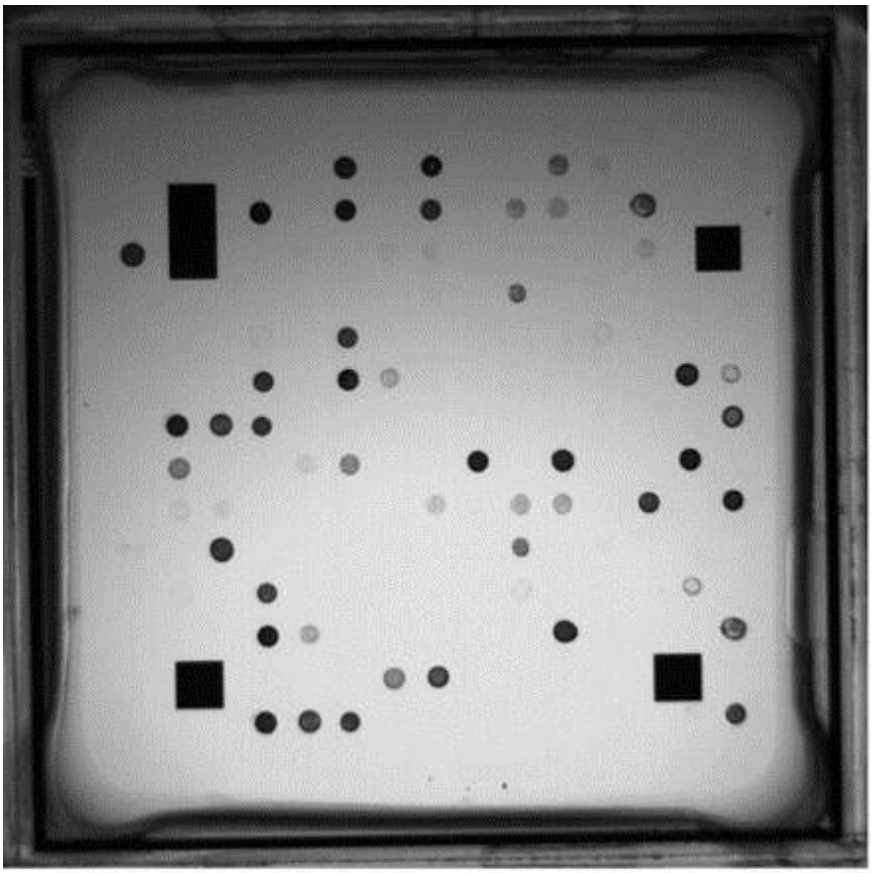

Plate 1. The tomato microarray.

Plate 2. The Genie II machine is capable of on-site testing using the LAMP assay.

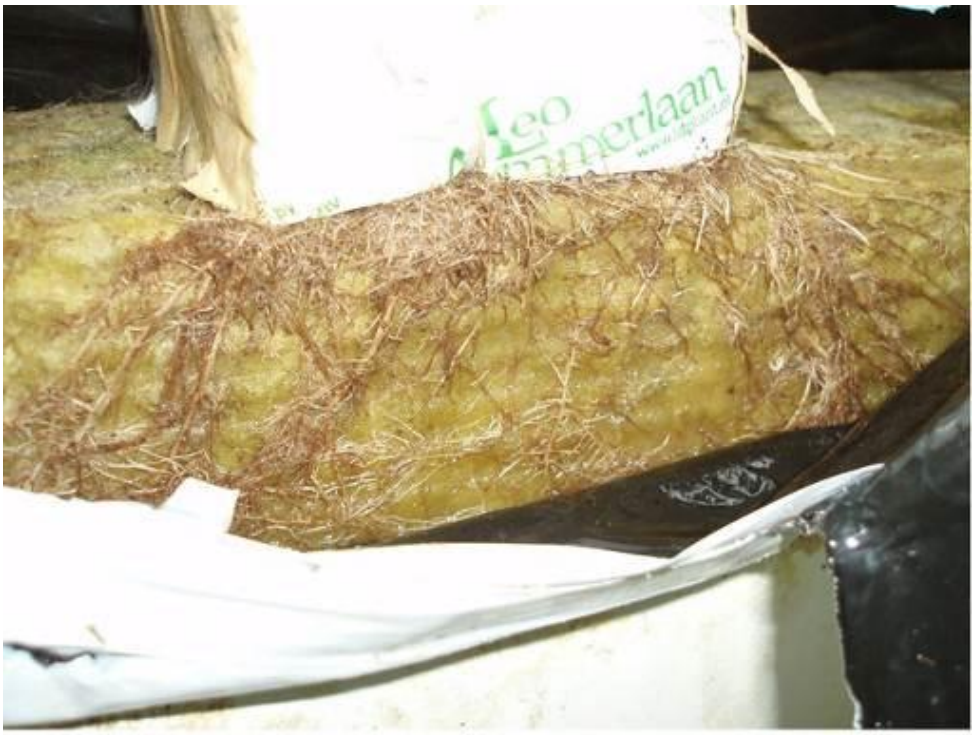

Plate 3. Pythium root rot in a commercial glasshouse.

Plate 4. On-site testing using the Genie II machine