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

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Grower Summary

Headline

Plant age, growing medium and disease occurrence have been shown to affect the diverse micro-organism population on tomato roots.

Background and expected deliverables

Non-pathogenic fungi and bacteria in the root environment (rhizosphere) can influence the occurrence of root disease. Obtaining information on the occurrence and levels of rhizosphere micro-organisms has, until recently, been difficult and time-consuming. A novel molecular method known as Terminal Restriction Fragment Length Polymorphism (T-RFLP) permits simultaneous identification and relative quantification of micro-organisms. This project used T-RFLP to investigate the micro-organisms associated with roots of tomato crops in various substrates. The expected deliverables from this project were:

1. An increased understanding of the role of rhizosphere micro-organisms in maintenance of root health;
2. Knowledge of whether a molecular test that determines occurrence and relative levels of different fungi and bacteria can be used to predict risk of root disease.

Summary of the project and main conclusions

Fungi and bacteria reported associated with tomato roots

From literature review, a large number of fungi and bacteria have been found associated with roots or growing media of tomato plants. A majority of these occur in the UK. Over 20 saprophytic fungi are recorded, including species associated with disease suppression by competing with or antagonising fungal pathogens. Mycorrhizal fungi associated with tomato roots are not well documented.

- At least 66 fungal pathogens (Table 1) and 4 bacterial pathogens have been reported associated with roots or growing media of tomato plants.
- Root disease development is influenced by biotic and abiotic factors; effects are often complex due to interactions and results are sometimes contradictory.

Table 1: Fungal pathogens reported previously on tomato roots

Fungus	Fungus
Alternaria solani	*Phymatotrichopsis omnivora*
Aphanomyces cladogamus	*Phytophthora* (18 species)
Botrytis cinerea	*Pyrenochaeta lycopersici*
Calyptella campanula	*Pyrenochaeta terrestris*
Collectotrichum coccodes	*Pythium* (19 species)
Didymella lycopersici	*Rhizoctonia solani*
Fusarium (7 species)	*Spongospora subterranean*
Humicola fuscoatra	*Thielaviopsis basicola*
Macrophomina phaseolina	*Verticillium* (5 species)
Monographella cucumerina	

Microbial populations on tomato roots in UK crops

In 2009 and 2010, the microbial populations associated with tomato roots were determined by T-RFLP analysis on 90 samples each year. These comprised three replicate samples of young roots collected from each of 10 commercial crops (two each grown on coir, rockwool or woodfibre slabs, in NFT solution or in soil) on three occasions during cropping. Most of the plants from which roots were sampled remained alive and healthy at the end of cropping but a few were dead or affected by Verticillium wilt, Fusarium wilt or crown and root rot or vascular staining. Black dot and black root rot were observed quite commonly on roots, especially in NFT solution and soil.

- Most micro-organisms identified by plating onto agar were also detected by T-RFLP analysis but T-RFLP detected many more micro-organisms.
- T-RFLP analysis indicated a tremendously wide range of fungi (over 100 species) and bacteria associated with tomato roots of the crops we sampled.

Potential fungal pathogens indicated by T-RFLP were found in all growing media, ranging in total from eight (NFT crops) to 13 species (soil crops) (Table 2).

The fungus *Plectosphaerella cucumerina* (*Fusarium tabacinum*)**,** a known cause of root and stem rot in tomato seedlings, was found in 17 of 20 crops and at abundant levels. The impact of this fungus on root growth during crop production warrants investigation.

Colletotrichum coccodes, the cause of black dot, was found in all growing media, in nine of the 20 crops and at relatively abundant levels, with increasing abundance from first fruit pick. Traditionally considered a weak pathogen that primarily affect plants near the end of cropping, these results may indicate an increasing problem.

Species of *Pythium* and *Fusarium* were each found in most growing media, sometimes at abundant levels. Pythium root rot and Fusarium crown and root rot were obvious in some of

the crops where these fungi were found. Varietal resistance, crop management and root environment likely influence why obvious root disease did not develop in the other crops.

Table 2: Potential fungal pathogens found associated with roots of 20 commercial tomato crops in England in 2009 and/or 2010 using T-RFLP

Potential fungal species	Disease common name	Crops detected in:		Relatively Abundant
		No. (of 20)	Growing Medium	
Botrytis cinerea	Grey mould	1	Coir	No
Colletotrichum coccodes	Black dot	9	All	Yes
Fusarium oxysporum	-	6	RW,soil,NFT,WF	No
Humicola fuscoatra	-	1	Coir	No
Macrophomina phaseolina	Charcoal rot	2	RW,soil	No
Plectosphaerella cucumerina	-	17	All	Yes
Phytophthora spp.	-	4	RW,coir,WF	No
Pyrenochaeta lycopersici	Corky root rot	4	Soil,coir,WF	Yes (soil,WF)
Pythium spp.	-	7	RW,soil,NFT,coir	Yes (soils)
Spongospora subterranea	Powdery scab	1	Coir	No
Thielaviopsis basicola	Black root rot	1	WF	Yes
Verticillium spp.	-	2	Soil	No

RW – rockwool; WF – woodfibre

Monitoring in successive years showed that certain root and vascular diseases occurred on the same nursery each year – notably **Fusarium crown and root rot, Pythium root rot and Verticillium wilt.** These results likely reflect variety choice/growing practices and possibly also the carryover of fungal pathogens on nurseries at crop turn-around.

Many likely saprophytic fungi were found in tomato roots including species of *Aspergillus, Cladosporium, Epiccocum, Gliocladium, Penicillium* and *Trichoderma*; the mycorrhizal fungi *Gigaspora* sp. was found in all substrates and at abundant levels. *Aspergillus, Penicillium, Gliocladium* sp. and *Trichoderma* sp. (potential antagonists) were found in most substrates.

T-RFLP was not very useful for investigation of bacteria associated with roots due to the occurrence of multiple potential identifications with many of the fragment lengths.

Pathogen presence and disease occurrence

T-RFLP tests on root samples collected during crop production detected, in total, 55 cases of likely root infection by 12 potential pathogens over the 20 crops (Table 2). In 12 of these cases the associated disease was confirmed at the end of cropping.

Potential fungal pathogens detected by T-RFLP which did not result in visible disease were *Humicola fuscoatra, Phytophthora* sp., *Plectosphaerella* sp., *Spongospora* sp. and *Macrophomina* sp. Some of these fungi are weak pathogens and may not have developed to levels sufficient to cause obvious disease.

In a few cases T-RFLP did not detect the fungi which were found to be causing disease in a crop – notably *Thielaviopsis basicola* in NFT crops and Verticillium wilt in coir crops.

Microbial diversity and disease prediction

Microbial population diversity on roots was examined. Plant and root health was assessed at the end of cropping and compared with microbial diversity on roots.

- Growth medium had a large effect on fungal population diversity, being least in NFT and greatest in soil.
- Fungal diversity increased progressively with time in rockwool, NFT and coir crops, but decreased with time in soil crops.
- Plant health and root rot at the end of the season was not associated with fungal or bacterial diversity indices determined earlier in cropping (i.e. from the results obtained in this work, T-RFLP is not useful as a tool for disease prediction).

Specific comparisons using T-RFLP

In plants with obvious root mat disease or Pythium root rot, fungal and bacterial species richness was greater, possibly a result of secondary colonisation by micro-organisms due to release of growth substrates from affected roots.

Examination of a suspension of Trianum P in water detected *T. harzianum* but the fungus was not detected on roots after injection into the rockwool crop nutrient solution.

Six rootstocks were compared in a soil-grown crop. Black dot was detected by T-RFLP at greatest abundance on Efialto and Optifort and a likely *Phytophthora* sp. was found only on Emperador and Unifort.

Effect of some growing medium amendments on microbial populations associated with tomato roots in soil

In 2010, an experiment was done to determine the effect of three pre-plant soil amendments (composted green waste, bark and Biofence) and two microbial drench treatments (Compete Plus in alternation with Colonize AG, and Trianum P) on plant survival and root health in an organic crop of cv. Piccolo on Beaufort rootstock.

Compared with previous years, the incidence of plant wilting and death was low. None of the treatments increased plant survival or decreased the incidence of vascular staining or root rot. *C. coccodes* and a *Fusarium* sp. were commonly isolated from roots.

T-RFLP showed that none of the treatments affected root microbial diversity. Around 15-25 fungi were identified on roots in each treatment. Predominant potential fungal pathogens were *C. coccodes, P. cucumerina, P. lycopersici* and *V. nigrescens*. *C. coccodes* levels increased greatly between first pick and July.

- These results indicate that the rhizosphere microbial population structure in soil grown tomato is not easily altered by the treatments we used.
- The occurrence of fungal pathogens on roots does not always lead to significant crop yellowing and plant death. However, they probably cause root loss.

Potential commercial test for root micro-organisms

T-RFLP testing is not currently offered as a commercial service and is not the ideal format for a rapid testing service. Molecular diagnostic methods have advanced greatly since this project started. Work is now being undertaken in two related projects at the University of Nottingham to refine the T-RFLP test and to develop additional tests, including a tomato rhizosphere fungal microarray. This can be probed with DNA extracted from tomato roots to provide more reliable information on species present and quantification data (Figure 1).

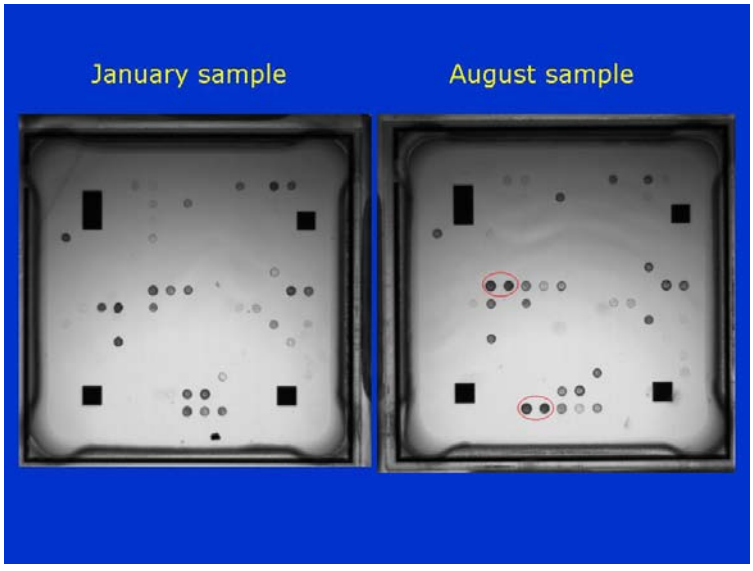

Figure 1. Tomato rhizosphere fungal microarray. DNA sequences from around 50 different fungi are on the plate, represented by 196 spots (2 sequences per fungus and 2 replicates per sequence). Colour development indicates presence of that fungus; intensity indicates relative amount. Early and late season root samples were taken from the same crop. The circled spots indicate increases of *Colletotrichum* and *Pythium* in the later samples.

Financial benefits

Estimates of tomato yield loss to root diseases in Britain have not been reported. Occurrence of Pythium root rot in rockwool and woodfibre crops on one nursery in 2009 is estimated to have cost over £50,000; occurrence of Verticillium wilt, corky root rot and black dot root rot in an organic crop is estimated to have cost £193,000. Yield loss due to root dieback associated with minor root pathogens is also likely to occur. With 145 ha of protected tomato in the UK in 2007 (Defra Horticultural statistics) and a farm gate value of £150 million (TGA estimate), and assuming 5% of marketable yield is lost due to root disease, this represents lost output valued at £7.5 million. If 10% of this loss could be prevented, the annual saving to growers would be around £1.5 million or £5,172/ha, less the cost of implementing the improved root disease control.

Action points for growers

- Growers should be aware of the range of diseases that can cause root loss, wilting and death of tomato plants (see Table 1).
- Growers should be aware that other potentially pathogenic fungi occur on tomato roots although their effect on plant health is uncertain. Such fungi found in this work include *Colletotrichum acutatum, Humicola fuscoatra, Macrophomina phaseolina* and *Plectosphaerella cucumerina*; the latter fungus was found to be very common and abundant on roots.
- Growers should check roots regularly (e.g. at least every 2 weeks) for evidence of root death or disease and, where found, identify the cause. Send a sample to a diagnostic laboratory or consult a plant pathologist when the cause of damage is unclear.
- Where a root disease has caused damage on a nursery, take particular care with clean-up, disinfection and maintenance of hygiene at crop turn-around. In the work done in this project, several nurseries affected by a specific root disease one year were found to have the same root disease the next year (e.g. Fusarium wilt, Verticillium wilt, root mat, Pythium root rot).
- A microarray that can check tomato roots for a wide range of fungal pathogens and beneficial micro-organisms in a single test is being developed. Growers should be aware that it is planned to test this on commercial crops in 2012.

Science Section

Root-infecting fungi are commonly found on the roots of tomato plants grown in nutrient film technique (NFT) and substrate crops, and occasionally cause severe disease. These include Pythium root rot, Phytophthora root rot and black root rot (*Thielaviopsis basicola*). Other pathogens occur less commonly, or are considered to be weakly pathogenic (e.g. black dot caused by *Colletotrichum coccodes*, root rot caused by some *Fusarium* species). Vascular wilt pathogens (e.g. *Fusarium oxysporum, Verticillium albo-atrum*) commonly infect plants via the roots, and Verticillium wilt caused by *V. albo-atrum* has been more troublesome since 1996. Additional root diseases have been recorded in soil-grown organic crops (e.g. brown and corky root rot, powdery scab), despite the use of disease-resistant rootstocks. It is difficult to monitor and quantify the development of root diseases due to the difficulty in viewing roots (except for NFT crops) and the need for laboratory tests to determine the precise cause. As a consequence, the early stages of a root disease are often missed.

A complex of fungi and bacteria live in, on and around tomato roots (the rhizosphere). Both plant pathogens and saprophytes, and culturable and non-culturable organisms are likely to be present. There is evidence that the presence of high populations of certain micro-organisms (e.g. *Aspergillus* spp., *Penicillium* spp., *Pseudomonas* spp.) can reduce disease through direct antagonism, the production of antimicrobial compounds, niche exclusion and/or stimulation of systemic plant defence responses. Interactions among pathogenic and non-pathogenic micro-organisms on roots are not well understood because of the difficulty and complexity of studying the root environment. If interactions among rhizosphere micro-organisms were better understood, it might be possible to better predict the risk of damaging root disease.

If it becomes obligatory to recycle waste irrigation solution from substrate crops, as has recently happened in Denmark, the potential for widespread root disease in a crop is significantly increased. Where recycling has been used in the UK previously, there were some significant disease problems (e.g. Phytophthora root rot). Several UK growers are now planning to recycle irrigation solution because of environmental concerns, increased fertiliser costs and the need to demonstrate sustainable systems.

With the recent move by Government and some retailers towards carbon-neutral sustainable production, there may be increased pressure to grow in organic media (e.g. bark and wood fibre products) rather than media that consume significant energy to produce, are used just once, and create a waste disposal problem (e.g. rockwool). The

microbial profile that develops on roots may differ considerably according to the medium in which a crop is grown.

The recently developed molecular technique of Terminal Restriction Fragment Length Polymorphism (T-RFLP), based on Polymerase Chain reaction (PCR) and DNA fragments analysis, allows semi-quantitative determination of fungal and bacterial genera in a single test. All fungi and bacteria associated with roots can be monitored and the relative amounts in different samples can be determined by using the plant DNA as an internal standard. Fungi and bacteria present in a sample are identified by reference to a database of the predicted DNA fragment sizes following PCR and treatment with specific restriction enzymes. This project aims to use T-RFLP to investigate the occurrence and relative levels of major pathogenic and non-pathogenic micro-organisms associated with roots of tomato crops. The information gained will be examined to determine if it can be used to predict the risk of root disease.

In Year 1 of the project we:

1. Compiled a list of fungi and bacteria previously found associated with tomato roots and briefly reviewed factors influencing root diseases;
2. Examined roots from tomato crops grown in soil, rockwool and NFT by culturing on agar and by T-RFLP;
3. Optimised a T-RFLP protocol for determining fungi and bacteria associated with tomato roots.

In Year 2 of the project we:

1. Identified fungi and bacteria by T-RFLP on roots of ten tomato crops over a growing season;
2. Assessed plants from the ten monitored crops for root health at the end of cropping;
3. Compared the populations of fungi and bacteria occurring on tomato roots from contrasting situations on individual nurseries;
4. Determined if T-RFLP results obtained during crop growth help to predict root health at the end of cropping;
5. Investigated the effect of some rhizosphere interventions on root health and microbial populations on roots.

The objectives in Year 3 were the same as in Year 2.

1. Effect of growing medium and crop age on microbial populations associated with tomato roots

Introduction

The development of root disease in tomato crops requires the presence of a pathogenic micro-organism. Once a pathogenic fungus or bacterium is present, key factors influencing disease development are pathogen inoculum level and populations of competing or antagonistic micro-organisms. In year 1, a molecular fingerprinting method (T-RFLP), based on DNA fragment length, was optimised for examination of microbial communities associated with tomato roots. The aim of the current work was to use T-RFLP to examine the effect of growing medium and crop age on microbial populations associated with tomato roots in commercial crops. Ultimately, the aim is to determine if T-RFLP or a similar method for monitoring microbial populations on roots, can be used to help predict risk of root disease in a crop, thereby allowing growers the opportunity to intervene before a serious root disease problem develops.

Materials and methods

Site and crop details

Ten commercial crops were examined, two each growing on rockwool slabs, in soil, in NFT solution, on coir slabs and on woodfibre slabs, using the same nurseries as in Year 2. Although it was not possible to use a common variety at all sites, the range of varieties used was kept to a minimum. Root samples from each crop were taken on three occasions: at 2-4 weeks after rooting into the growing medium, around first pick and in peak production. Crop details and dates of root sampling are given in Table 1.1.

Table 1.1: Details of tomato crops monitored in 2010

Growing medium and site code	Variety	Date planted	Dates sampled			Final assess
			Early	Mid	Late	
Rockwool						
1.	Star	Mid Dec	29 Jan	20 Apr	17 Aug	25 Oct
2.	Encore	Early Jan	1 Feb	19 Apr	18 Aug	27 Oct
Soil						
3.	Roterno*	Early Feb	9 Feb	11 May	10 Aug	15 Oct
4.	Piccolo*	End Jan	31 Mar	26 May	25 Aug	15 Oct
NFT						
5.	Encore	Mid Dec	22 Jan	8 Apr	missing	25 Oct
6.	Anamay	End Dec	15 Apr	6 Jul	05 Oct	25 Oct
Coir						
7.	Encore	Mid Jan	25 Feb	20 Apr	5 Aug	21 Oct
8.	Dometica+	Mid Jan	17 Mar	10 May	9 Sep	9 Nov
Woodfibre						
9.	Star	Mid Dec	29 Jan	20 Apr	17 Aug	25 Oct
10.	Cheramy	End Jan	10 Mar	18 May	17 Aug	15 Oct

* On Beaufort rootstock

\+ On Emperador rootstock

Root samples

At each visit, root samples were collected from three plants in one row. Each sample was split into three sub-samples in the laboratory to provide nine microbial population profiles. Sampled plants were labelled and adjacent plants in the same row were used at sequential visits. Young roots were collected from beneath the cube (visit 1) or at the corner of a slab (visits 2 and 3); by forking away soil for soil crops; or by cutting off root tips (visit 1), or midway between plants (visit 2 and 3), in NFT crops. A minimum of 2 g root fresh weight was collected from each plant. Samples were stored at 4ºC until transfer to Nottingham University, or were posted directly.

Solution samples

For crops grown on rockwool, coir or woodfibre slabs, waste irrigation solution (50 mL) was collected from the base of slabs using a new plastic syringe. For the NFT crops, solution was taken from the channel. No solution samples were collected from soil crops.

Crop assessments

At each visit, the three plants from which root samples were taken were examined for leaf yellowing, wilting, stem disease and root appearance.

Towards the end of cropping, all nine plants were assessed for plant health (alive or dead), vascular staining in the stem base, and root appearance. Additionally in 2010, the number of dead stem bases and dead or wilting heads was assessed on the full row length or 100 plants; vascular staining in the stem base was assessed on 20 green stem bases. Where there was obvious root decay or discolouration, samples of roots were examined in the laboratory by microscopy and/or by culturing on agar to determine the identity of fungi associated with different symptoms. Plants in the same row as monitored plants and with symptoms of poor growth attributable to root disease were also examined as above to determine identity of fungi associated with roots. Dates of the final crop assessment are given in Table 1.1.

DNA extraction and T-RFLP testing

The methods described in the Year 2 report were followed.

Reference cultures

Cultures were acquired from various sources, including ADAS and Fera. Fungal cultures were kept on Potato Dextrose Agar (Oxoid, Basingstoke, UK) and bacteria on Nutrient Agar (Oxoid). All cultures were stored at 4^{o}C. Isolates were analysed by sequencing the 28S ribosomal gene or ITS2 region and by T-RFLP sequence analysis.

Analysis

Low quality peaks (<200 relative fluorescent units) were removed from the analysis, as well as peaks equating to <1% of the total microbial population. Peaks which were less than 1.5 bp apart from a larger peak (shoulder peaks) were eliminated and their peaks added to the larger peak.

FRAGSORT

To help identify qualifying T-RFLP peaks, the resulting data was normalized and imported into a peak resolving program called FRAGSORT (Michel and Sciarini, 2003). The software resolves T-RFLP profiles by attempting to maximize the matches between the peaks in the profiles and the entries in the fungal and bacterial database, so that the number of peaks

left without a matching sequence is minimal. The software output only shows those sequences which have their terminal restriction fragments (TRFs) in both profiles cut with the two enzymes.

Simpson's diversity index (Equation 1) was used to characterize species diversity among samples (Simpson, 1949). It takes into account the number of species present, as well as the abundance of each species. In addition, the Simpson diversity index is relatively insensitive to under-sampling (Chao and Shen, 2003). The value of this index ranges between 0 and 1, the greater the value, the greater the sample diversity.

$$D = 1 - \left[\frac{\Sigma n(n-1)}{N(N-1)}\right]$$

Equation 1: Simpson's Diversity Index (1 – **D).**

Where: N = the total number of organisms of all species;
n = the total number of organisms of a particular species.

Principal component analysis (PCA) was carried out on T-RFLP data, which had been transformed into relative abundance data, using Genstat 12. The covariance matrix was used for this type of data, as recommended by numerous papers (Ramette, 2007; Culman *et al*., 2008). PCA provides a means to separate and group samples based on their community patterns, since it simultaneously considers many correlated variables and then identifies the lowest number to accurately represent the structure of the data.

Results and discussion

Plant and root health

Very few of the sampled plants showed symptoms of poor growth during the season (Table 1.2). The exception was one soil crop (site 4) which showed leaf yellowing and necrosis at the later visits; one NFT crop (site 5) which showed leaf yellowing and discoloured roots in August; one coir crop (site 7) which showed leaf yellowing and poor roots in September.

At the end of cropping, all monitored plants were alive except for one plant in a rockwool crop at site 2 (Fusarium wilt or stem rot), one plant in an NFT crop at site 6 (due to Fusarium crown and root rot) and one plant in a coir crop at site 8 (due to Verticillium wilt) (Table 1.3).

Root blackening was obvious on the mass of fine roots in NFT crops, and *Colletotrichum coccodes* and/or *Thielaviopsis basicola* was confirmed associated with these symptoms. Root blackening was also obvious in the soil crop at site 3, largely due to black dot (*C.*

coccodes). A high density of fine roots was present in rockwool, coir and woodfibre slabs, and in the NFT solutions, whereas plants in the two soil crops had few fine roots. No root mat symptoms were seen on any plants. The fungi found associated with roots or stem base of plants by microscope examination and culture tests are summarised in Table 1.4.

The number of dead or missing stem bases in the monitored row towards the end of cropping ranged from zero (sites 8 and 10) to 20% (site 7, a coir crop affected by Verticillium wilt and stem Botrytis) (Table 1.5). The incidence of plants with vascular staining in the stem base, an indicator of likely root and/or vascular wilt disease, was zero in two crops (one NFT and one woodfibre) and was 20% or greater in three crops (the two soil crops, and an NFT crop affected by Fusarium crown and root rot). The *Fusarium* species isolated from stem bases at site 6 was confirmed as *Fusarium oxysporum* by a molecular test (Dez Barbara, University of Warwick); from the symptoms and varietal susceptibility (cv. Anamay) to Fusarium crown and root rot it was concluded to be *F. oxysporum* f. sp. r*adicis-lycopersici*.

Table 1.2: Summary of visual health of plants sampled for routine root monitoring – 2010

Site No.	Growing medium	Sample occasion	Number of plants (of 3) affected by:						
			Leaf yellow	Leaf wilt	Leaf necrosis	Stem disease	Leaf disease	Roots not white	Root rots/spots
1.	Rockwool	1	1	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	3	0
2.	Rockwool	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0
3.	Soil	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0
4.	Soil	1	0	0	0	0	0	0	0
		2	3	0	1	0	0	0	0
		3	1	0	3	0	0	0	0
5.	NFT	1	0	0	0	0	0	0	0
		2	0	0	3	0	0	3	0
		3	Missing (data not supplied)						
6.	NFT	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	2	0
7.	Coir	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	3	0
8.	Coir	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0
9.	Woodfibre	1	0	1	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0
10.	Woodfibre	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0

See Table 1.1 for sample dates.

Table 1.3: Summary of stem base and root assessments on 9 monitored plants at end of cropping – 2010

Site	Growing medium	Number of plants (of 9)				Mean severity on roots (0-3)			Density of fine roots (0-3)
		Alive	*Verticillium* sporing	*Fusarium* sporing	Root mat	Root black/ black dot	Roots brown/ decayed	Corky roots	
1.	Rockwool	9	0	0	0	1	2	0	3
2.	Rockwool	8	0	3	0	1	2	0	3
3.	Soil	9	0	0	0	2	2	1	1
4.	Soil	9	0	0	0	1	1	1	1
5.	NFT	9	0	0	0	2	0	0	3
6.	NFT	8	0	1	0	2	0	0	3
7.	Coir	8	1	0	0	0	1	0	3
8.	Coir	9	0	0	0	0	1	0	3
9.	Woodfibre	9	0	0	0	0	1	0	3
10.	Woodfibre	9	0	0	0	0	1	0	3

Table 1.4: Summary of fungal pathogens found associated with nine plants during routine root monitoring or at the end of cropping by isolation onto agar and/or microscopy - 2010

Site	Growing medium	*Colletotrichum coccodes*	*Fusarium* spp.	*Pyrenochaeta lycopersici*	*Pythium* sp.	*Thielaviopsis basicola*	*Verticillium* sp.
1.	Rockwool	✓●■	✓■		●■		■
2.	Rockwool		✓●		✓●		
3.	Soil	✓●■	■	✓●■	●■		●
4.	Soil	✓	✓	✓	✓●		✓●
5.	NFT	✓			✓	✓	
6.	NFT	●	✓ ●*		✓●	✓	
7.	Coir		✓		✓●		✓
8.	Coir						
9.	Woodfibre	✓●	✓	●	✓		
10.	Woodfibre		●			●	

Trichoderma sp. was also recovered from roots at sites 1 and 4.

Sites 1 and 3 only were also analysed using a microarray test for tomato root fungi.

✓ Identified using classical plating methods ● Identified by T-RFLP ■ Identified by Microarray

* confirmed as *F. oxysporum* by a DNA test (D Barbara, University of Warwick), and considered to be Fusarium crown and root rot from the symptoms and that the variety, cv. Anamay, does not have genetic resistance to this pathogen.

Table 1.5: Summary of stem assessments in monitored rows (c. 100 plants) at end of cropping – 2010

Site	Growing medium	% plants with:		
		Dead/missing stem base	Dead or wilted head	Vascular browning in stem base[a]
1.	Rockwool	2	0	5
2.	Rockwool	11	11	10
3.	Soil	7	12	20
4.	Soil	17	17	35
5,	NFT	3	3	0
6.	NFT	6	9	65
7.	Coir	20	32	5
8.	Coir	0	0	5
9.	Woodfibre	2	4	15
10.	Woodfibre	0	0	0

[a] Assessed on 20 green stem bases.

Association of microbial diversity on roots with plant health

Occurrence of dead plants, vascular browning in the stem base of live plants, and root decay and discolouration were assessed in this work and used to calculate a 'plant sickness' score (range 0-18; based on numbers of dead plants and vascular staining in the stem) and a 'root rot' score (range 0-12; based on numbers of plants with decay or discolouration or corkiness of major and minor roots) (Table 1.6). Scores were determined by assessing the numbers of plants with different symptoms and using a weighting factor (x2) for the most severe symptoms (dead plants and decay of major roots; see Table 1.6).

Data for 2009 and 2010 were combined and examined by regression analysis to provide 20 data sets (Table 1.7). There was no obvious association between either 'plant sickness' or 'root rot' scores and fungal diversity or bacterial diversity (as measured by the Simpson diversity index) at any of the sampling times (Table 1.8). There appeared to be an association between plant sickness and bacterial diversity at the T2 sample time, but this was likely due to one low bacterial diversity value (0.475) which corresponded to a zero in plant sickness score. This was for the NFT data set which had the extremes for bacterial diversity and influenced the result at this time point.

Overall, the lack of obvious association between either 'plant sickness' or 'root rot' scores and fungal diversity or bacterial diversity may be due to the limited data set, the difficulty in objectively determining root health, the use of different varieties and growing media, and the

complexity of potential microbial interactions on roots. Ideally this aspect of the work should have focussed on one growing medium and one variety in order to reduce confounding variation. Work elsewhere has shown that plant variety can influence rhizosphere micro-organisms and plant health (e.g. Tucci *et al*., 2011). However, the project steering group requested work be done on all the main growing media, and there were inevitably different varieties being grown at different nurseries. Future work seeking to relate microbial diversity with root health should, wherever possible, focus on one variety and one growing medium.

Table 1.6: Plant and root health at the end of cropping in 10 tomato crops – 2010

Assessment	RW		Soil		NFT		Coir		WF	
	1	2	3	4	5	6	7	8	9	10
Incidence										
Number plants dead (of 9)	0	1	0	0	0	1	1	0	0	0
Incidence of stem vascular browning [a]	0.5	1	2	3.5	0	6.5	0.5	0.5	1.5	0
Number with visible stem *Verticillium*	0	0	0	0	0	0	1	0	0	0
Number with visible stem *Fusarium*	0	3	0	0	0	1	0	0	0	0
Severity (0-3)										
Major roots decayed/brown	2	2	2	1	0	0	1	1	1	1
Minor roots brown or black	1	1	2	1	2	2	0	0	0	0
Corky roots present	0	0	1	1	0	0	0	0	0	0
Plant and root health (based on the 9 monitored plants)										
Plant sickness score (2 x no. dead + no. with vascular brown) (0 – 18)	1	3	2	3.5	0	8.5	2.5	0.5	1.5	0
Root rot score (2 x major root decay + no. minor root decay+ no. corky) (0 – 12)	5	5	7	4	2	2	2	2	2	2

RW - rockwool; WF- Woodfibre.

[a] Estimated value for 9 plants based on assessment of the whole row.

Table 1.7: Crop and root appearance and fungal (F) and bacterial (B) diversity at three time points (T1, T2, T3) during crop production in 20 tomato crops

Crop No.	Crop type	Year sampled	Plant sickness (0-18)	Root rot (0-12)	Microbial diversity (0-1)					
					F-T1	F-T2	F-T3	B-T1	B-T2	B-T3
1	RW	2009	9	3	0.5021	0.6408	0.5751	0.8485	0.8358	0.8112
2	RW	2009	5	0	0.4088	0.3059	0.5316	0.8182	0.8536	0.8248
3	Soil	2009	14	9	0.7442	0.6997	0.6984	0.8937	0.8295	0.8524
4	Soil	2009	13	11	0.7837	0.7714	0.7505	0.8259	0.8162	0.8611
5	NFT	2009	3	2	0.3905	0.6921	0.7385	0.8711	0.6453	0.8874
6	NFT	2009	7	2	0.0862	0.0239	0.3245	0.7011	0.9055	0.8505
7	Coir	2009	14	6	0.3419	0.3715	0.4179	0.8306	0.7915	0.8085
8	Coir	2009	10	0	0.4817	0.6449	0.6085	0.8287	0.7734	0.8466
9	WF	2009	9	5	0.5514	0.6200	0.3238	0.6617	0.9009	0.8136
10	WF	2009	10	1	0.4910	0.5267	0.5481	0.8546	0.8622	0.8854
11	RW	2010	1	5	0.6503	0.4975	0.7113	0.8773	0.7692	0.8532
12	RW	2010	3	5	0.4975	0.6828	0.8108	0.8590	0.8574	0.8484
13	Soil	2010	2	7	0.7491	0.7770	0.6198	0.8801	0.8440	0.8605
14	Soil	2010	3.5	4	0.6894	0.7965	0.6976	0.8383	0.8778	0.7599
15	NFT	2010	0	2	0.6256	0.5669	0.4689	0.8328	0.4754	0.8240
16	NFT	2010	8.5	2	0.7510	0.6370	0.7270	0.8017	0.7810	0.7285
17	Coir	2010	2.5	2	0.6958	0.6968	0.7944	0.8531	0.8982	0.8905
18	Coir	2010	0.5	2	0.7421	0.6196	0.8439	0.8683	0.8930	0.8472
19	WF	2010	1.5	2	0.6850	0.7867	0.7264	0.8837	0.8102	0.8959
20	WF	2010	0	2	0.7111	0.7501	0.7670	0.9125	0.8674	0.8513

Table 1.8: Association of microbial diversity on tomato roots at three time points during crop production with crop and root appearance at the end of cropping (data for 2009 and 2010 combined; n=20)

Crop assessment	% variance accounted for in relation of fungal (F) and bacterial (B) diversity with crop appearance at three sample times (T1, soon after planting; T2 first fruit; T3 mid August)					
	F1	F2	F3	B1	B2	B3
Plant sickness	13	0	22	0	72	0
Root rot	0	3	0	29	0	0

Fungal pathogens and saprophytes in individual crops

A summary of likely fungal pathogens detected in each of the 10 crops as determined by T-RFLP tests on crops during the season, is given in Table 1.9. A total of 15 potential pathogens were found. As in 2009, *P. cucumerina* was the most common fungus, occurring in all 10 crops and at relatively high abundance levels. Other fungi detected frequently were *Colletotrichum* spp. (7 crops) and *Pythium* spp. (3 crops).

The number of potential fungal pathogens in the different growing media was broadly the same ranging from seven (rockwool and woodfibre) to 10 (coir). Data on potential fungal pathogens detected over the two years (20 crops) is shown in Table 1.10. These results illustrate that in all growing media roots are susceptible to infection by a range of fungal pathogens.

A large number of saprophytic fungal species were indicated by T-RFLP (Appendix 1). As in 2009, the mycorrhizal fungus *Gigaspora rosae* and species of *Aspergillus* and *Penicillium* were detected most frequently (Table 1.11). Species of *Gliocladium* and *Trichoderma* were each detected in three crops. Some species of the fungal genera found, especially *Gliocladium* and *Trichoderma,* are recognised antagonists of certain fungal pathogens.

Table 1.9: Occurrence and minimum relative abundance of possible fungal pathogens associated with tomato roots as determined by T-RFLP analysis of root samples collected during crop production – 2010

Fungal species	Growing medium and crop									
	RW		Soil		NFT		Coir		Woodfibre	
	1	2	3	4	5	6	7	8	9	10
Botrytis cinerea	-	-	-	-	-	-	-	0.15	-	-
Calyptella sp.	-	-	0.41	-	-	1.81	-	0.23	-	-
Colletotrichum acutatum	-	-	1.66	16.58	-	0.74	1.79	0.37	-	-
Colletotrichum coccodes	2.89	-	-	-	-	2.59	-	-	2.92	-
Cylindrocarpon destructans	-	4.08	-	-	-	0.16	-	-	-	1.40
Fusarium sp.	-	4.08	-	-	-	0.16	-	-	-	1.40
Humicola fuscoatra	-	-	-	-	-	0.95	-	-	-	-
Macrophomina phaseolina	-	-	0.35	-	-	-	-	-	-	-
Phytophthora sp.	-	-	-	-	-	-	-	0.15	-	-
Plectosphaerella spp.	21.84	19.22	4.57	11.93	6.09	19.49	1.73	4.31	5.46	0.49
Pyrenochaeta lycopersici	-	-	0.35	-	-	-	-	-	4.03	-
Pythium spp.	1.63	0.39	-	-	-	-	0.42	0.15	-	-
Spongospora subterranean	-	-	-	-	-	-	1.01	-	-	-
Thielaviopsis basicola	-	-	-	-	-	-	-	-	-	10.32
Verticillium sp.	-	-	1.40	0.53	-	-	-	-	-	-

Table 1.10: Potential fungal pathogens detected in roots of 20 tomato crops by T-RFLP according to substrate – 2009/2010

Fungus	Number of crops (of 4) where fungus detected:					
	RW	Soil	NFT	Coir	WF	Total
Botrytis cinerea	0	0	0	1	0	0
Colletotrichum spp.	2	3	2	4	0	11
Fusarium spp.	2	1	1	0	2	6
Humicola fuscoatra	0	0	1	1	0	2
Macrophomina spp.	1	1	0	0	0	2
Phytophthora spp.	1	0	0	1	2	4
Plectosphaerella spp.	4	3	4	3	3	17
Pyrenochaeta sp.	0	1	0	0	1	2
Pythium spp.	2	1	0	2	0	5
Spongospora subterranea	0	0	0	1	0	1
Thielaviopsis basicola	0	0	0	0	1	1
Verticillium spp.	0	3	0	0	0	3
Total	12	13	8	13	9	55

Table 1.11: Likely fungal saprophytes associated with roots of commercial tomato crops in different substrates – 2010

Fungal genus	RW 1	RW 2	Soil 3	Soil 4	NFT 5	NFT 6	Coir 7	Coir 8	WF 9	WF 10
Aspergillus spp.	✓			✓		✓	✓	✓	✓	
Cladosporium spp.		✓		✓			✓			
Epicoccum spp.	✓									
Gigaspora spp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Gliocladium spp.		✓				✓				✓
Glomus spp.						✓				
Penicillium spp.			✓	✓		✓	✓	✓	✓	✓
Trichoderma spp.				✓			✓			✓

Association of T-RFLP results with root infection and disease

A direct comparison of T-RFLP results with root microscopy and/or crop disease observations was not possible as tests were done on different root samples collected at different stages of crop production. Assuming that the root samples collected at each stage were representative of the crops, the results of T-RFLP tests done on samples collected

during crop production were compared with those of root microscopy and crop observations at end of crop production (Table 1.12).

There was agreement between T-RFLP and microscope observations in nine out of 31 cases of potential root infections detected across the 10 crops. T-RFLP indicated 15 potential pathogens (Table 1.9) whereas microscopy and culturing revealed six.

Results for the two years (20 crops) are summarised in Table 1.13 for eleven potential pathogens. T-RFLP and microscopy indicate the same likely root infection in 12 of 44 cases. T-RFLP detected a wider range of potential pathogens than microscopy. In some instances, T-RFLP tests did not detect fungi observed in roots, notably *T. basicola* in NFT crops and *Verticillium* sp. in coir crops. The former was likely due to confusion of TRF length with *C. coccodes*; the latter may have been due to late season infection in the crops.

Overall, the T-RFLP results indicated a greater occurrence of root infection than was seen as visible disease. This is likely due to: some root infecting fungi causing minor root loss which does not necessarily result in crop symptoms (e.g. *Pythium, Colletotrichum, Plectosphaerella*); low inoculum levels of a fungus do not always results in root infection; control of fungal pathogens by antagonists; ability of a variety to tolerate a low level of infection; or other reasons.

The effect of low levels of root infection by strong pathogens and common infection by weak pathogens (e.g. *Plectosphaerella*) on crop yield is unknown. As plants will use metabolic energy to resist infection and produce new roots, potential crop yield must be reduced.

Table 1.12. Potential fungal pathogens detected in 10 tomato crops by T-RFLP roots tests (T) and/or microscopy (M) for pathogens observed microscopically in at least one crop – 2010

Fungal genus	Rockwool		Soil		NFT		Coir		WF	
	1	2	3	4	5	6	7	8	9	10
Colletotrichum spp.	TM	-	TM	TM	-	T	T	T	-	-
Fusarium spp.	M	TM	-	M	M	TM	M	-	M	T
Pyrenochaeta	-	-	TM	M	-	-	-	-	T	-
Pythium	T	TM	-	M	M	M	TM	T	M	-
Verticillium	-	-	T	TM	-	-	M	-	-	-
Thielaviopsis	-	-	-	-	M	M	-	-	-	T

T-RFLP tests were done on samples collected during cropping; microscopy was done on sample collected at end of cropping

Table 1.13: Potential fungal pathogens detected in tomato roots by T-RFLP on roots sampled during crop production and found by microscopy examination at the end of crop production – 2009/10

Fungus	Number of crops (of 20) with fungus detected by:		
	T-RFLP only	Microscopy only	Both
Colletotrichum sp.	7	1	4
Fusarium sp.	3	9	3
Humicola fuscoatra	2	0	0
Macrophomina sp.	2	0	0
Phytophthora sp.	4	0	0
Plectosphaerella sp.	17[a]	-	-
Pythium spp.	3	4	2
Pyrenochaeta sp.	2	2	2
Spongospora sp.	1	0	0
Thielaviopsis sp.	1	4	0
Verticillium sp.	2	4	1
Total	44	24	12

[a] Classed as *Fusarium* sp. in microscope examination.

Microbial population diversity

As in 2009, Simpson's diversity index and Principal Component Analysis (PCA) were used to examine data.

From fungal and bacterial relative abundance data, Simpson's diversity index values indicated that neither growing medium nor sample time had a significant effect on microbial diversity ($P > 0.05$). Fungal diversity index values ranged from 0.5 (rockwool, early sample) to 0.8 (coir, late sample) (Figure 1.1). Bacterial diversity indices were all above 0.8 except for the mid and late season NFT crops (Figure 1.2).

These results are in contrast to 2009, when growing medium and crop age significantly influenced fungal diversity. However, when the 2010 data were examined by PCA, significant effects of growing medium and crop age on fungal diversity were revealed (Figures 1.3 – 1.4). The soil and coir fungal diversity differed from NFT, coir and woodfibre (Figure 1.3); and the early season fungal diversity differed from mid and late season (Figure 1.4). Two fungal fragments that contributed to these differences were identified: *Colletotrichum* sp. (high levels in soil and woodfibre crops; and in mid and late samplings)

and *Plectosphaerella cucumerina* (high in NFT and coir crops; and at the mid-samplings) (Table 1.14).

Growing media and sample time also had a significant effect on the bacterial community (Figures 1.5 – 1.6). NFT crops had a distinctive bacterial community compared with the four other growing media (Figure 1.5); and the early sampled roots had a distinctive bacterial community compared with the mid and late season sampled roots. One bacterial fragment that contributed to these differences was identified, as likely *Methylobacterium* sp., which was present at high levels in the soil, coir and wood fibre crops (compared with NFT and rockwool), and at early and late sample times (Table 1.15).

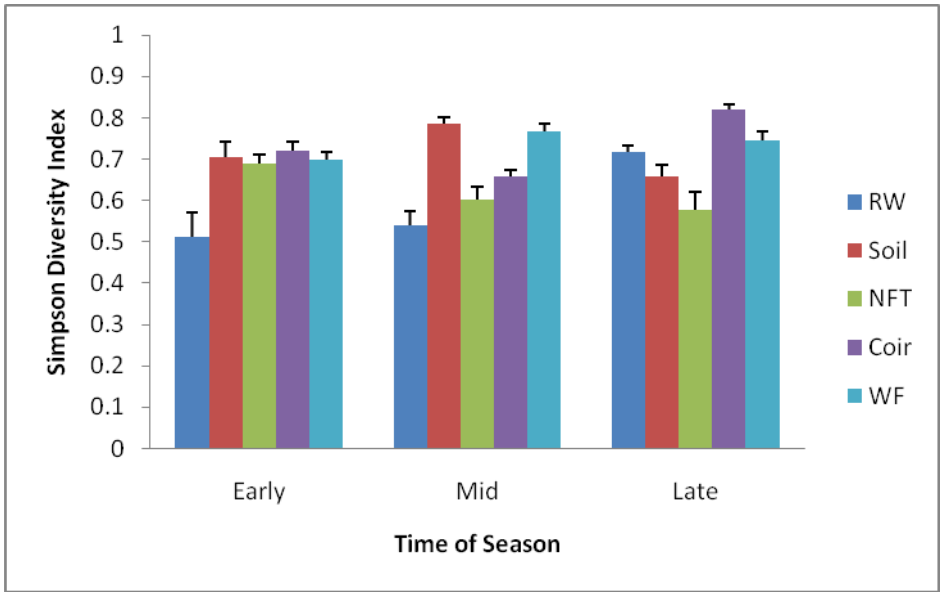

Figure 1.1. Effect of sampling time (plant age) and growth medium on fungal diversity calculated using Simpson's diversity index for 10 crops at 3 sampling times in 2010

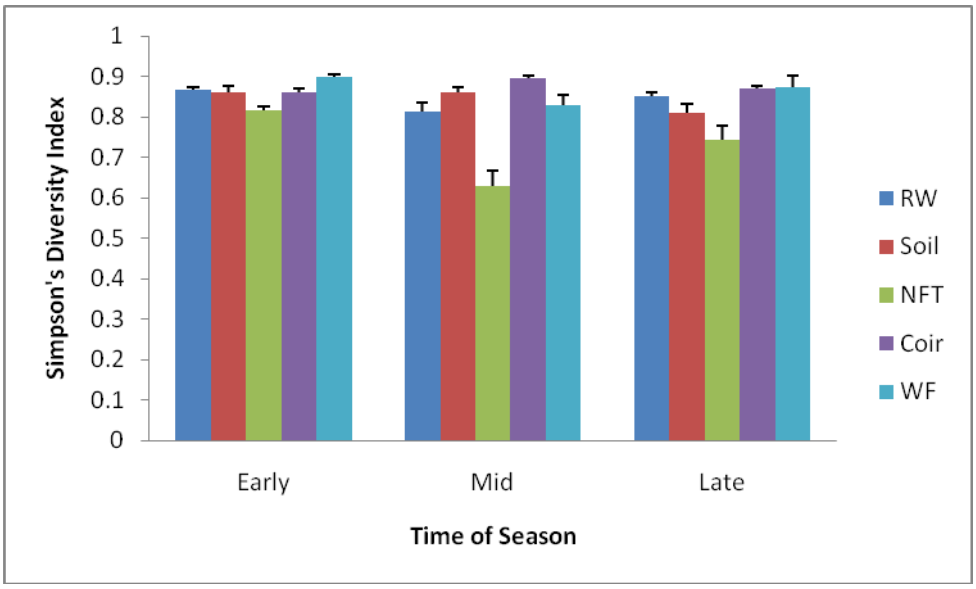

Figure1.2. Effect of sample time (plant age) and growth medium on bacterial diversity calculated using Simpson's diversity index for 10 crops at 3 sample times in 2010.

The occurrence of pathogenic and beneficial micro-organisms in soilless culture of greenhouse tomato was recently reviewed (Vallance *et al*., 2010). It was reported that once plants are introduced into glasshouses, growing media are rapidly colonised by bacteria and fungi. Our results support this report. Inorganic substrates were reported mainly colonised by bacteria, and organic substrates by fungi. We found large numbers of both fungal and bacterial species in both classes of substrate. Fluorescent Pseudomonads were reported higher on tomato in rockwool than in peat substrates, and the reverse was true for fungi, actinomyces and *Trichoderma* spp. In our work, T-RFLP rarely detected *Pseudomonas* spp. in rockwool or other hydroponic media. It seems likely this was due to failure of our T-RFLP test to detect this bacterial species.

Vallance *et al*. (2009) reported that microbial communities of tomato plants grown hydroponically for 6 months increased in complexity and size of fungal microflora as the season progressed. Our results generally support this view.

Plectosporium sp. (syn. *Plectosphaerella cucumerina*) (*Fusarium tabacinum*) was frequently isolated from roots of tomato crops grown in soilless culture in France (Blancard, unpublished data) and considered its pathogenicity to tomato in hydroponic systems needs to be assessed. We also found this species in nearly all crops and at relatively abundant levels.

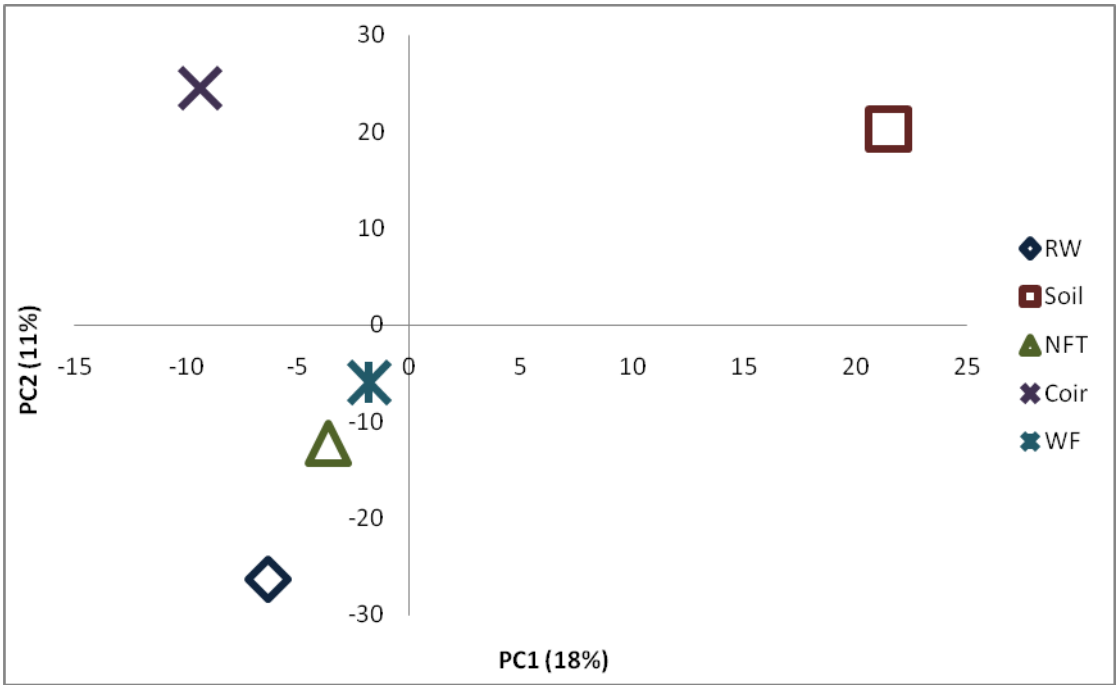

Figure 1.3. Ordination plot of PC1 versus PC2 for fungal relative abundance data from routine sampling of 10 crops – effect of growth media - 2010

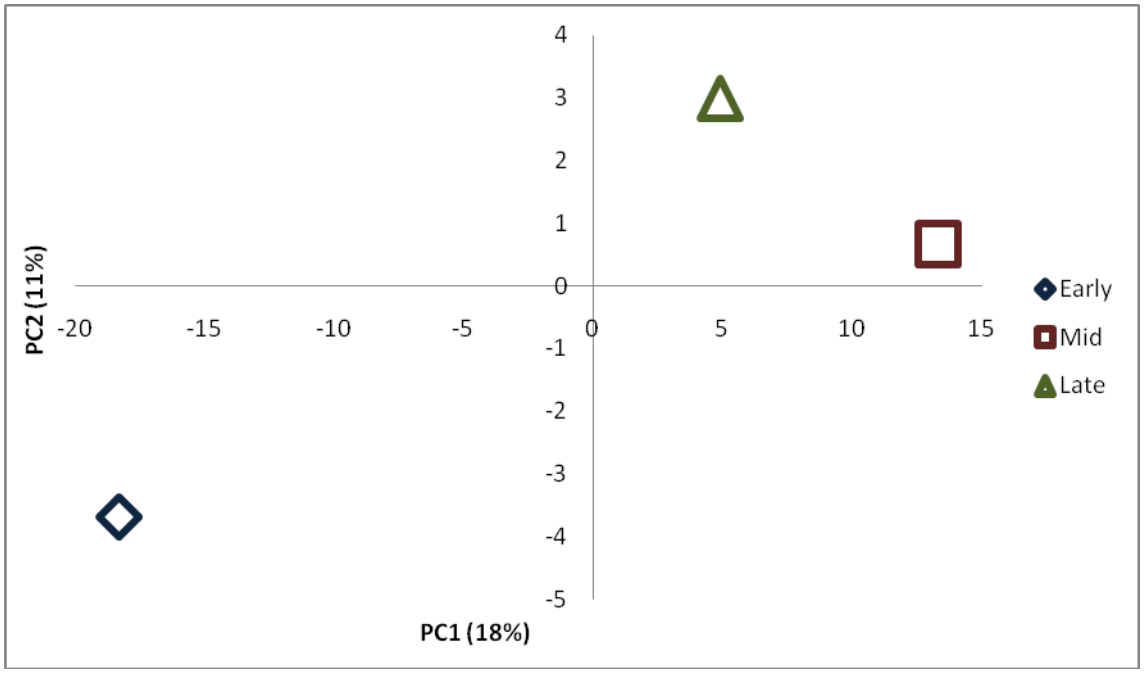

Figure 1.4. Ordination plot of PC1 vs PC2 for fungal relative abundance data from routine sampling of 10 crops – effect of sampling time - 2010

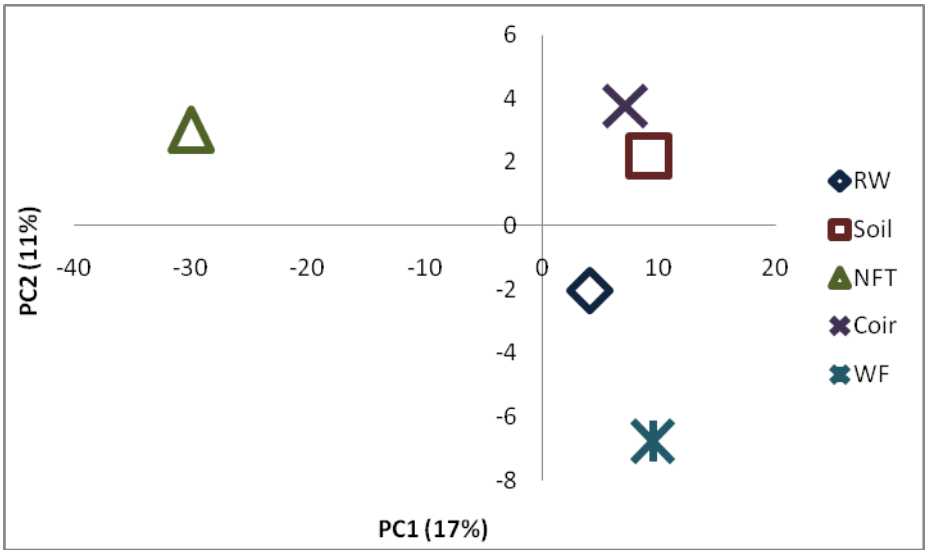

Figure 1.5. Ordination plot of PC1 versus PC2 for bacterial relative abundance data from routine sampling of 10 crops – effect of growth medium - 2010

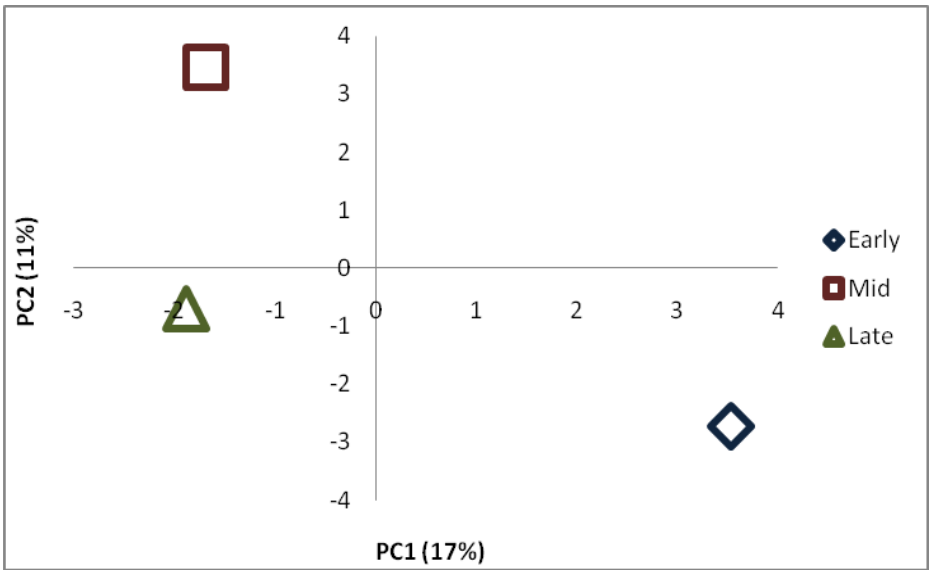

Figure 1.6. Ordination plot of PC1 versus PC2 for bacterial relative abundance data from routine sampling of 10 crops – effect of sample time - 2010

Table 1.14: Fragments making a significant contribution to PC1, PC2 and PC3 and their possible identities (H – cut with enzyme Hae III; M – cut with enzyme MseI)

Fragment Length/Enzyme	PC1	PC2	PC3	Potential Identity	Media	Time
FLA_324	-	0.41489	-	Unknown	S/W	M/L
FLA_341	0.32806	-0.27024	-0.34618	*Plectosphaerella cucumerina*	ALL	ALL
FLA_384	-0.75064	-	-0.38021	Unknown	R/N/C/W	E/M
FLH_154	-	0.48548	-	*Colletotrichum spp.*	S/W	M/L
FLH_138	-	-	-0.44706	*Plectosphaerella cucumerina*	N/C	M
FLH_205	-0.39479	-0.31813	-	Unknown	R/N/C/W	E

Table 1.15: Fragments making a significant contribution to PC1, PC2 and PC3 and their possible identified (H – cut with enzyme Hae III; M – cut with enzyme MseI)

Fragment Length/Enzyme	PC1	PC2	PC3	Potential Identity	Media	Time
FLH_151	-	-	-0.3862	Unknown	R/N	M
FLH_201	-	-	0.40741	*Methylobacterium sp. / Agrobacterium radiobacter 3813*	S/C/W	E/L
FLH_375	-0.36722	-0.64827	0.2815	Unknown	ALL	ALL
FLM_151	-	-	-0.37033	Unknown	R/N	M
FLM_340	-	-	0.2481	*Methylobacterium sp.*	S/C/W	E/L

2. Effect of some specific factors on microbial populations

Introduction

Various factors are likely to influence the populations of micro-organisms associated with tomato roots including the use of microbial amendments, age of slab, disinfestation of recycled solution and the presence of root disease. The aim of this work was to determine the effect of presence or absence of some individual factors on the microbial populations associated with tomato roots.

Materials and methods

Site and crop details

Samples were taken from commercial crops as opportunities for specific factor comparisons arose during 2009 and 2010. Details of the crops sampled and factors examined are given in Table 2.1.

Table 2.1: Details of crops sampled for comparison of the effect of individual factors on populations of micro-organisms associated with tomato roots – 2009 and 2010

Treatment comparison	Growing medium	Variety	Date sampled
1. Root mat absent, slight or severe	Rockwool	Not recorded	July 2010
2. [a]Compete Plus monthly drench vs none	Ekofibre	Cheramy	25/05/09
3. UV vs untreated NFT solution	NFT	Aranka	July 2010

[a] A root zone inoculant marketed by Plant Health Care including *Bacillus, Pseudomonas, Streptomyces* and *Trichoderma*, formulated with vitamins, humic acids and seaweed extract.

Root and solution samples

Samples were collected as described previously. For each paired comparison, three samples of each level (e.g. treated/untreated) were collected on one occasion, usually from plants in the same row. Three sub-samples from each were examined by T-RFLP (i.e. nine T-RFLP profiles per factor).

Root mat

Preliminary work was done to confirm the TRF lengths produced when *Agrobacterium radiobacter*, vector of the Ri plasmid that causes root mat disease, was examined by T-

RFLP. Twelve cultures of *A. radiobacter* isolated from tomato or cucumber plants affected by root mat disease were received from Fera (Table 2.1.1). The 23s ribosomal region of the strains was sequenced to determine their relationship (Figure 2.1.1) and to ensure that all strains were detected when examined by T-RFLP (Table 2.1.2). Root samples showing nil, slight and severe root mat symptoms were collected from the same row of a rockwool crop.

Table 2.1.1: Detail of *Agrobacterium radiobacter* strains examined by DNA sequencing

Protect No	Organism name	Host	Country
3478	*A. radiobacter*	Tomato	UK
3555	*A. radiobacter*	Tomato	UK
3475	*A. radiobacter*	Tomato	UK
3576	*A. radiobacter*	Tomato	UK
3813	*A. radiobacter*	Tomato	UK
4143	*A. radiobacter*	Tomato	Spain
5013	*A. radiobacter*	Cucumber	France
6322	*A. radiobacter*	Cucumber	UK
6371	*A. radiobacter*	Cucumber	UK
6392	*A. radiobacter*	Tomato	UK
6399*	*A. radiobacter*	Tomato	UK
6994	*A. radiobacter*	Tomato	UK

* Reported to be strongly pathogenic.

UV treatment of NFT solution

Six root samples were collected from the same glasshouse, three from plants in UV treated water and three from plants in non-UV treated water in July 2010. Roots were examined for micro-organisms by culture onto agar and by T-RFLP.

Four 1 cm long root samples were cut from all plants and plated on PDA; half were surface sterilized in sodium hypochlorite (1% for 1 minute). Individual cultures were sub-cultured onto new plates. Clean cultures were PCR amplified, cleaned up and sequenced.

Results and discussion

2.1 Root mat

The relationship of *Agrobacterium* isolates to each other is shown in Figure 2.1. The fragment lengths resulting from treatment of the different isolates are shown in Table 2.1.2.

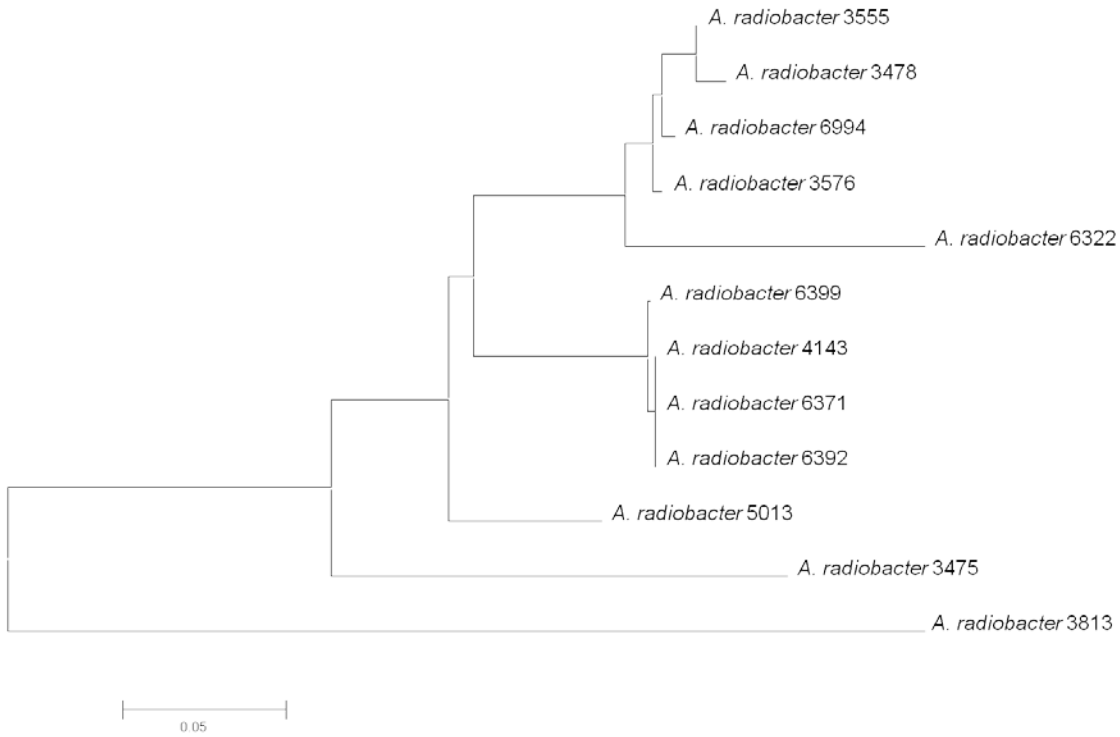

Figure 2.1.1. Phylogenetic tree of 23s ribosomal region of 12 *A. radiobacter* strains.

Table 2.1.2: Fragment lengths of 12 *A. radiobacter* strains when cut with two enzymes, Hae III (H) and MseI (M)

Strain reference no.		H157	H168	H202	M360	M371	M388	M472	M517	M633
A. radiobacter	3478	1			1					
A. radiobacter	3555	1			1					
A. radiobacter	3475			1					1	
A. radiobacter	3576	1			1					
A. radiobacter	3813		1			1				
A. radiobacter	4143	1								1
A. radiobacter	5013	1						1		
A. radiobacter	6322	1					1			
A. radiobacter	6371	1								1
A. radiobacter	6392	1								1
A. radiobacter	6399	1								1
A. radiobacter	6994	1			1					

Microbial diversity and species richness

Disease stage significantly affected both fungal and bacterial diversity (P= <0.05, P= <0.01 respectively). Fungal diversity increased with root mat disease symptom expression whereas bacterial diversity decreased (Figure 2.1.2).

Disease stage also significantly affected fungal and bacterial species 'richness' (P= <0.05, P= <0.01, respectively). Fungal species richness was significantly higher in early disease symptoms than in healthy. Bacterial species richness was significantly higher at early disease symptoms stage (Figure 2.1.3).

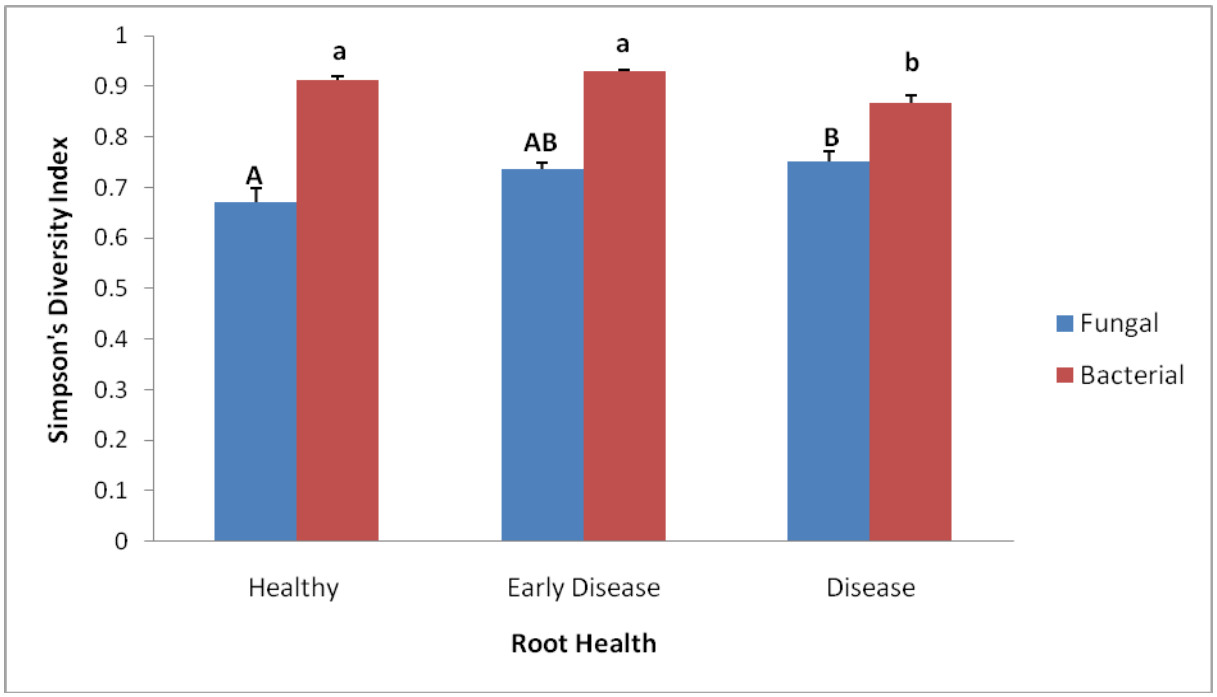

Figure 2.1.2. Effect of root mat disease stage on microbial diversity calculated using Simpson's Diversity Index. Error bars represent 1 standard error from the mean. Letters in common indicate samples are not significantly different.

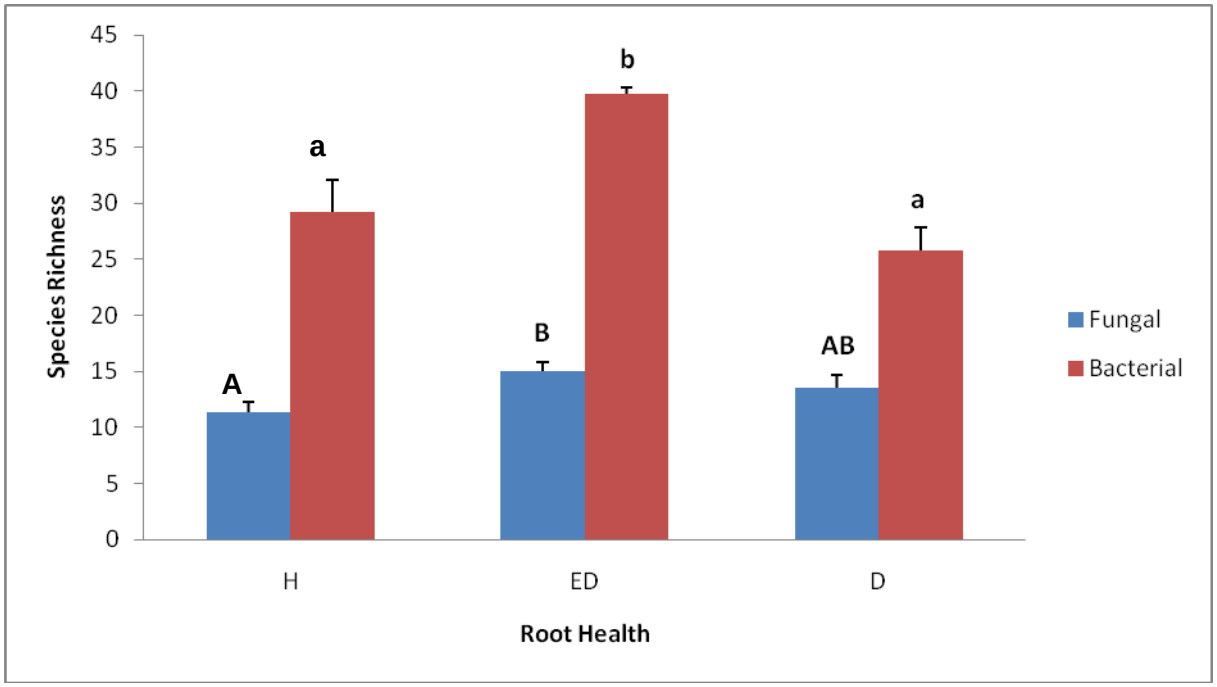

Figure 2.1.3. Effect of root mat disease stage on microbial species richness calculated using Simpson's Diversity Index. Error bars represent 1 standard error from the mean. Letters in common indicate samples are not significantly different.

Relative abundance of *A. radiobacter*

T-RFLP picked up at least two strains (3813 and one other) of *A. radiobacter* on late disease, early disease symptoms and healthy roots alike (Table 2.1.3). Strain 3813 was originally isolated from a crop in the UK.

Early diseased roots had significantly lower relative abundance levels of *A. radiobacter* than those of healthy and severely diseased roots, this could be explained by higher diversity and species richness competing with *A. radiobacter* (Figures 2.1.4 – 2.1.5).

Table 2.1.3: Detection of *A. radiobacter* strains in tomato roots visibly healthy or with early or late symptoms of root mat disease

Strain	Enzyme		Relative abundance (%)		
	Hae III	MseI	Healthy	Early Symptoms	Disease
Agrobacterium radiobacter 3478	157	360	8.85869	6.62003	9.47686
Agrobacterium radiobacter 3555	157	360	8.85869	6.62003	9.47686
Agrobacterium radiobacter 3576	157	360	8.85869	6.62003	9.47686
Agrobacterium radiobacter 3813	202	371	9.53575	6.58966	12.45396
Agrobacterium radiobacter 6944	157	360	8.85869	6.62003	9.47686

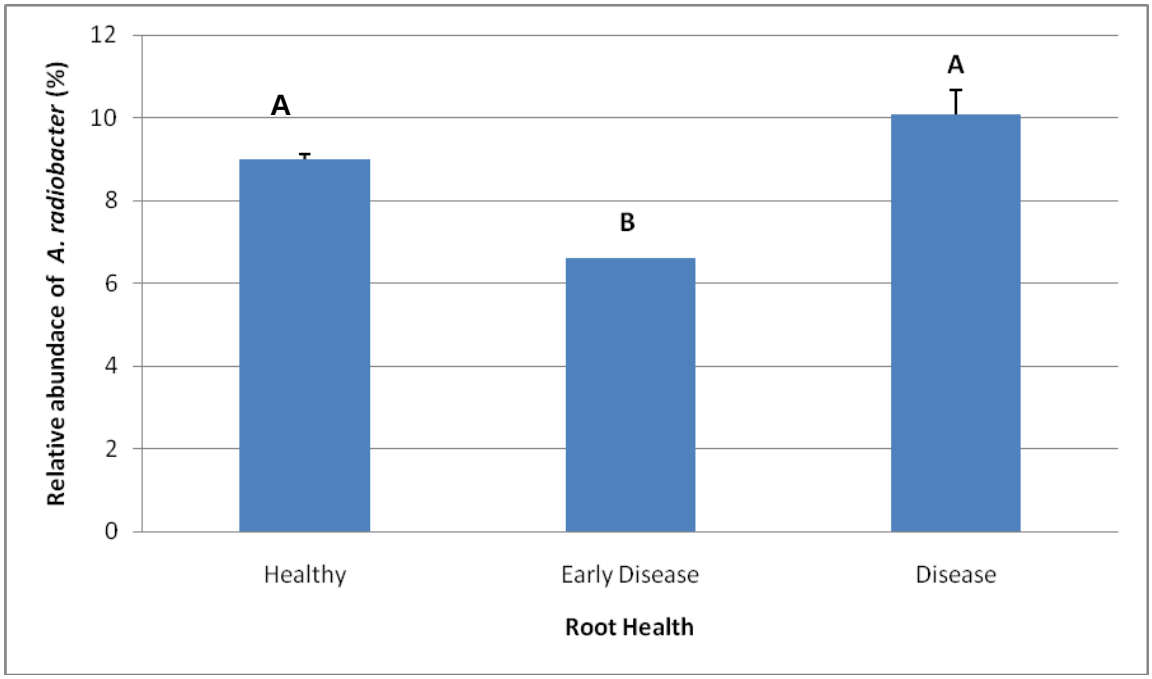

Figure 2.1.4. Mean relative abundance (%) of *A. radiobacter* on visibly healthy roots, early disease symptoms and confirmed/late disease. Error bars represent 1 standard error from the mean. Letters in common indicate samples are not significantly different.

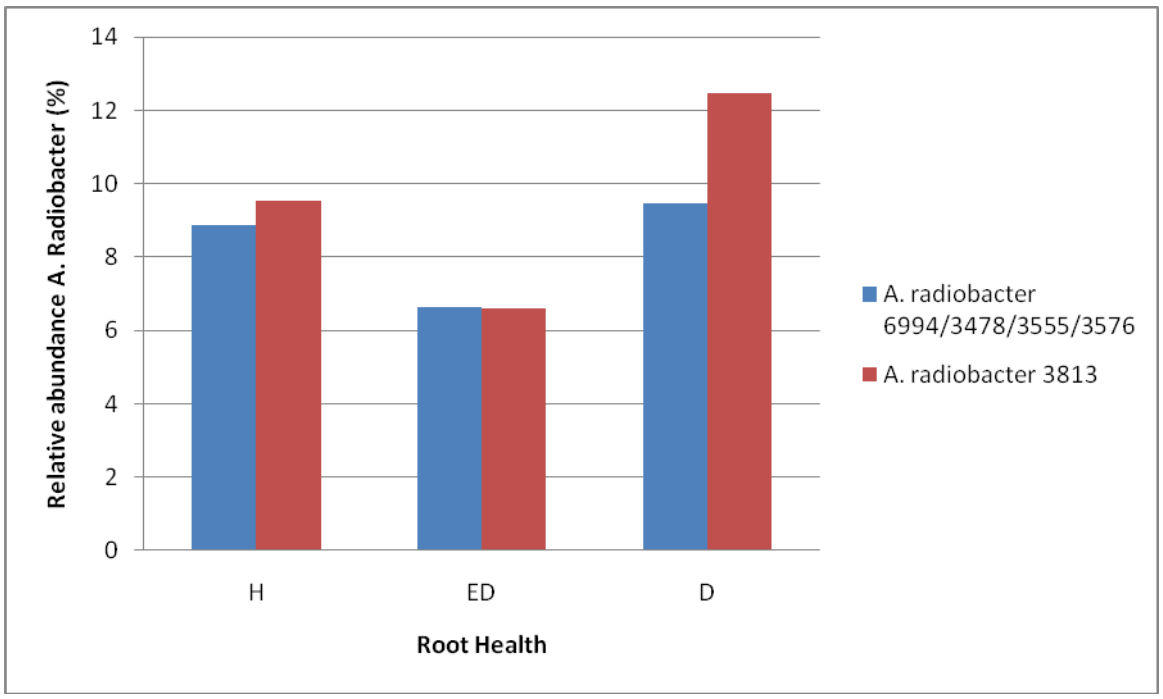

Figure 2.1.5. Minimum mean relative abundance of two potential strains of *A. radiobacter* on visibly healthy roots, early disease symptoms and confirmed/late disease.

Principal Component Analysis

Principal component analysis (PCA) using the covariance matrix was carried out on the relative abundance data of all T-RFLP fragment profiles, but analyzing bacteria and fungi profiles separately.

Principal component 1 (PC1) accounted for 56% of the total variation within samples, PC2 accounted for 16 % and PC3 accounted for 8% of the fungal relative abundance data set. These first three principal components explained a total variation of 80%, providing good coverage of the data (Table 2.1.4).

Table 2.1.4: Results of principal component analysis of fungal T-RFLP data from three stages of root mat disease

Principal component	PC1	PC2	PC3
Variation (%)	55.97	16.00	7.87
Cumulative (%)	55.97	71.97	79.84

An Anova of PC scores showed that PC1 and PC3 were significantly different between the three root mat disease stages (P= <0.01), however PC2 was not significant (P=0.4).

Fungal community

The relative abundance of fungi differed between healthy; early and late root mat disease symptoms (Figures 2.1.6 – 2.1.7 and Table 2.1.5). From looking at the table of significant peaks and the ordination plot it can be seen that *Plectosphaerella cucumerina* and *Fusarium oxysporum* levels are higher, contributing to the diseased fungal community. This is evident since both *Plectosphaerella cucumerina* and *Fusarium oxysporum* have significant positive PC scores (0.32, 0.25 respectively) to PC1 and on the ordination plot late root mat disease PC scores are positive.

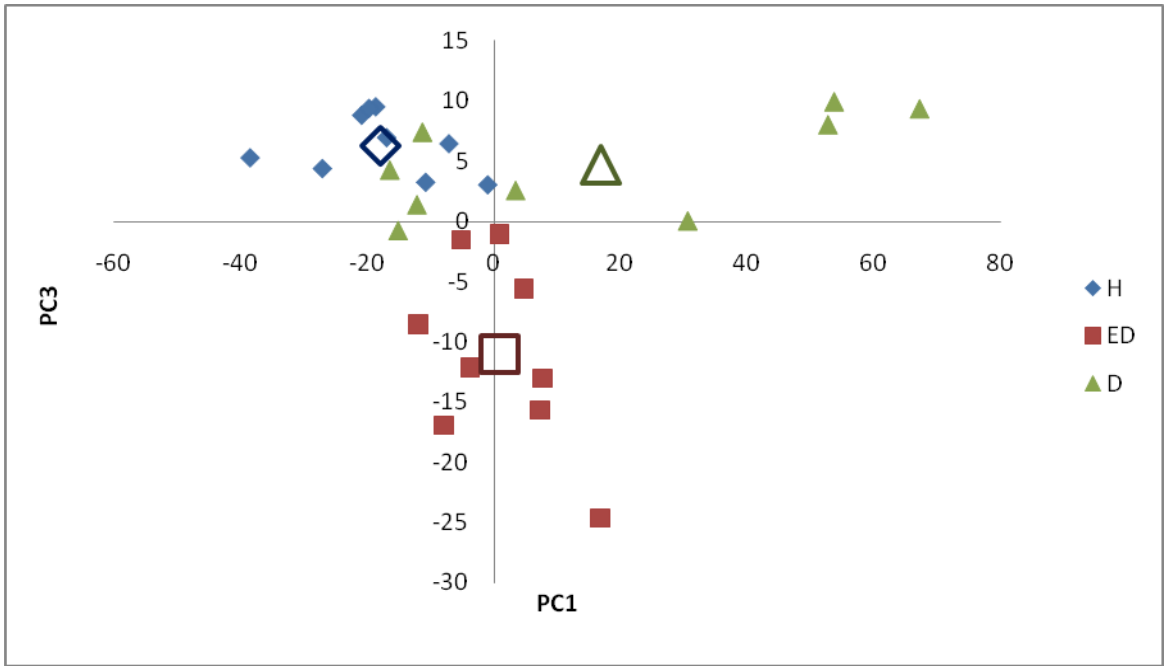

Figure 2.1.6. Ordination plot of PC1 versus PC3 scores using fungal relative abundance data of healthy (H), early disease symptoms (ED) and late root mat disease.

Note: Large symbols represent mean PC score centroids.

Table 2.1.5: Significant fragment lengths contributing to PC1 and PC3 and their possible identity

Fragment Length/Enzyme	PC1	PC3	Potential Identity
FLA_342	0.32	-	*Plectosphaerella cucumerina*
FLA_383	-	-0.44	Unknown
FLH_73	0.25	-	*Fusarium oxysporum*
FLH_183	-	0.39	Unknown
FLH_206	-	-0.30	Unknown

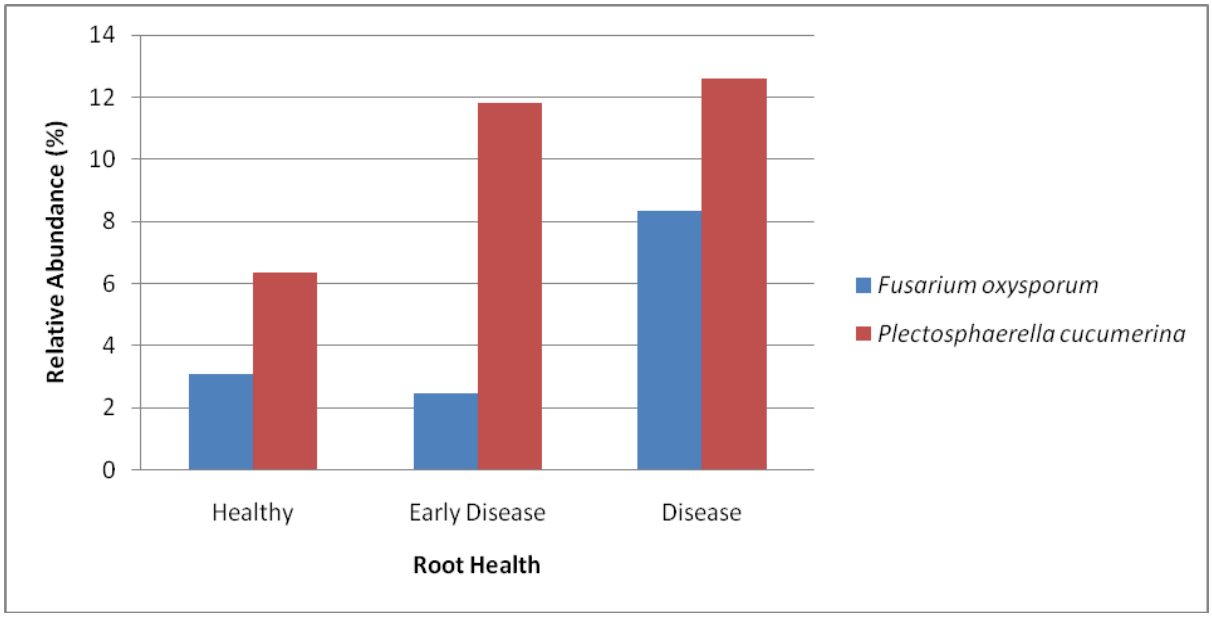

Figure 2.1.7. Relative abundance levels of fungal organisms significantly different between three root mat disease stages when using PCA analysis.

Bacterial community

The first three principal components accounted for 81% of the total bacterial variation, providing good coverage of the data (Table 2.1.6). From looking at the table of significant peaks (Table 2.1.7) and the ordination plot (Figure 2.1.8) it can be seen that all three identified peaks are more abundant/significantly contributing to the diseased bacterial community.

Table 2.1.6: Percentage variation of bacterial T-RFLP data from three stages of root mat disease

Principal component	1	2	3
Variation (%)	50.76	22.32	7.68
Cumulative (%)	50.76	73.08	80.76

An Anova of PC scores showed that PC1 was significant between the three root mat disease stages (P= <0.01), however PC2 and PC3 were not significant (P=0.2, P=0.4 respectively).

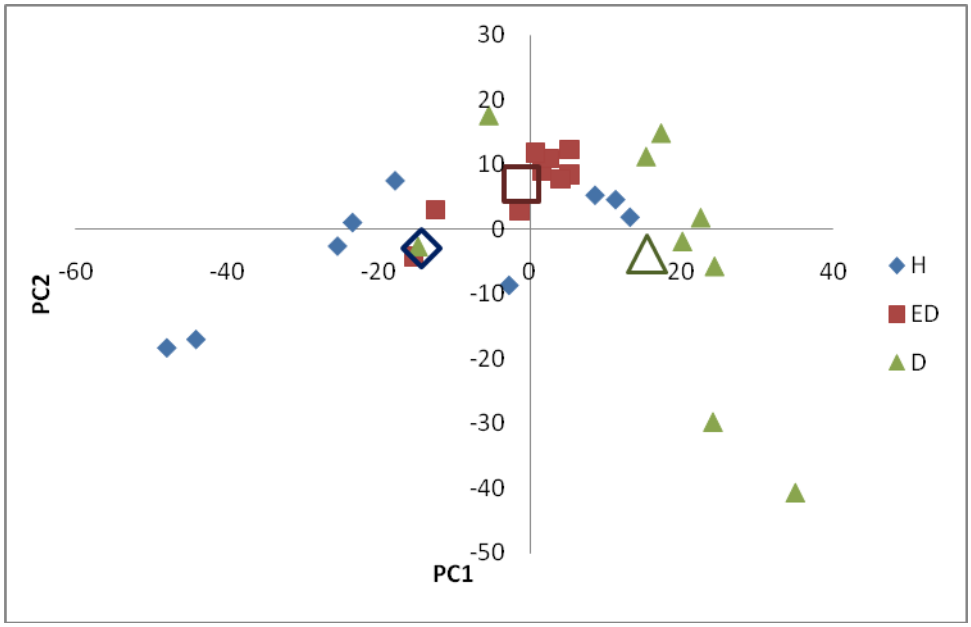

Figure 2.1.8. Ordination plot of PC1 versus PC2 scores using bacterial relative abundance data of healthy (H), early disease symptoms (ED) and late root mat disease.

Note: Large symbols represent mean PC score centroids.

Table 2.1.7: Significant fragment lengths contributing to PC1

Fragment Length/Enzyme	PC1	Potential Identity
FLH_202	0.78277	Unknown
FLH_400	0.32999	Unknown
FLM_400	0.36441	Unknown

2.2 Compete™ Plus drench treatment

Compete™ Plus (CP)

Compete™ Plus (available from Plant Health Care) label information indicates that it contains bacteria such as *Bacillus, Pseudomonas*, and the actinomycete *Streptomyces*, as well as beneficial soil fungi of the genus *Trichoderma*. These microbes are formulated in a proprietary nutrient blend of vitamins, soluble humic acids, and seaweed extract.

T-RFLP of neat product in water identified seven fungi and 16 bacteria. The majority of the micro-organisms present were not identified. One fungal constituent composes 30% of the total community (FLH_170 and FLA_226) however it is not in the current database and therefore has not been identified. Attempts to clone the micro-organisms present in Compete™ Plus have been unsuccessful due to low levels of DNA from extractions.

Bacterial constituents identified using FRAGSORT included plant growth promoting organisms and bacteria involved in nitrogen fixation.

Compete ™Plus vs. untreated control (2009)

The effect of three drenches of Compete™ Plus (CP), at monthly intervals, on a woodfibre tomato crop was examined. Bacterial diversity and species richness were significantly higher on the roots treated with CP (P= <0.05, P= *<0.01* respectively). There was no significant difference in the fungal diversity or species richness levels between CP and no amendment (NA) control (P= 0.9, P= 0.4) (Figures 2.2.1 and 2.2.2).

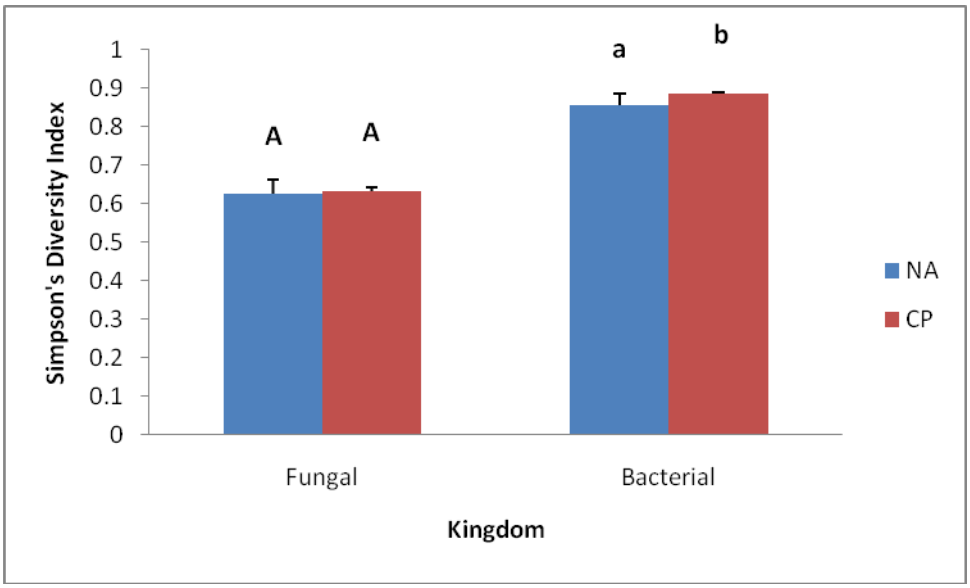

Figure 2.2.1. Effect of Compete™ Plus (CP) treatment on microbial diversity of the rhizosphere compared with no amendment (NA) control calculated using Simpson's Diversity Index. Error bars represent 1 standard error from the mean. Treatments with a letter in common are not significantly different.

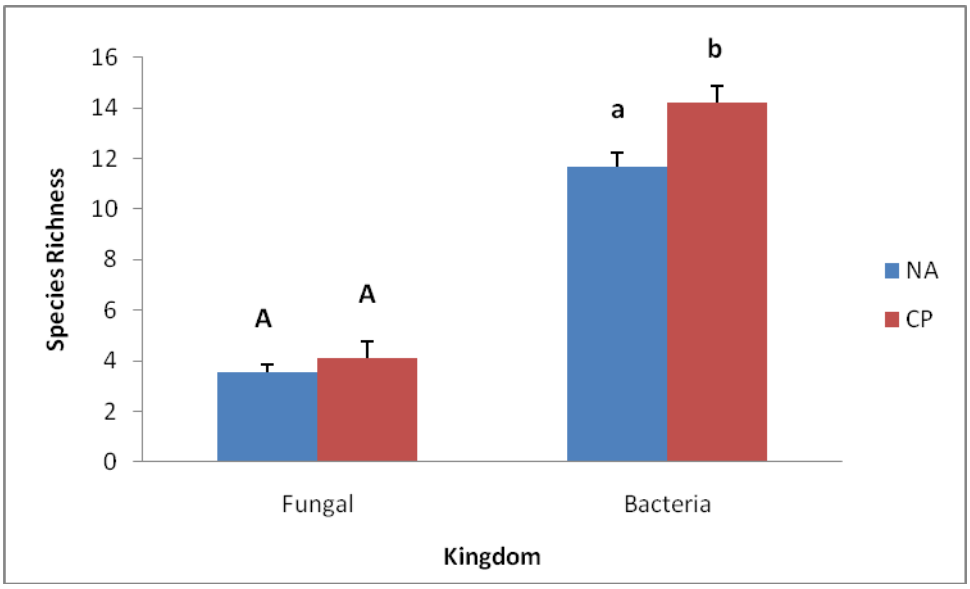

Figure 2.2.2. Effect of Compete™ Plus (CP) treatment on the species richness of the rhizosphere, compared with a no amendment (NA) control. Error bars represent 1 standard error from the mean. Treatments with a letter in common are not significantly different.

Populations were then examined by Principal Component Analysis.

Fungal community

Principal component 1 (PC1) accounted for 51% of the total variation within CP and NA samples, PC2 accounted for 27% and PC3 accounted for 18% of the fungal relative abundance data set. These first three principal components explained a total variation of 97%, providing excellent coverage of the data (Table 2.2.3).

Table 2.2.3: Results of principal component analysis of fungal T-RFLP data from CP treated and NA control crops.

Principal Component	PC1	PC2	PC3
Variation (%)	51.13	27.27	18.17
Cumulative (%)	51.13	78.40	96.57

An Anova of PC scores showed that PC1 and PC3 were significant between CP and NA samples (P= <0.05, P= <0.01 respectively), however PC2 was not significant (P= 0.07).

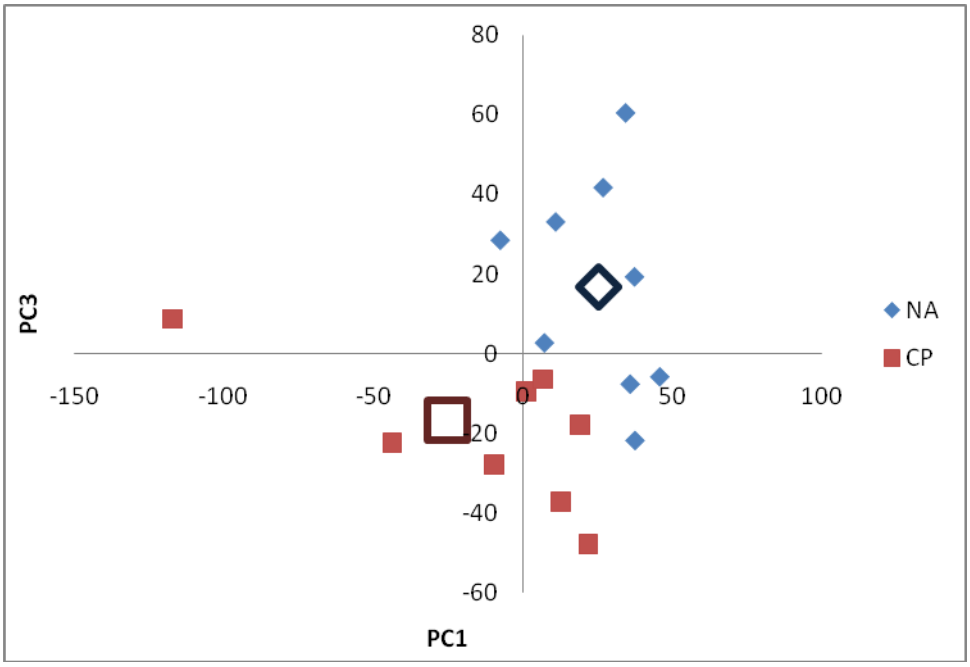

Figure 2.2.3. Ordination plot of PC1 versus PC3 scores using fungal relative abundance data of Compete™ Plus (CP) treatment and no amendment (NA) control plants.

Note: Large symbols represent mean PC score centroids.

Table 2.2.4: Significant fragment lengths contributing to PC1 and PC3, their possible identity and whether they are associated with CP or NA samples

Fragment Length/Enzyme	PC1	PC3	Potential Identity	CP vs. NA
FLA_181	-	0.45181	Unknown	NA
FLA_343	0.36672	-	*Plectosphaerella cucumerina*	NA
FLA_384	-0.5864	-	Unknown	CP
FLH_138	0.25724	-	Unknown	NA
FLH_153	-	-0.66189	*Colletotrichum coccodes*	CP
FLH_206	-0.60827	-	Unknown	CP
FLH_343	-	0.44797	*Gigaspora rosae*	NA

From looking at the table of significant fragments (Table 2.2.4) and the ordination plot (Figure 2.2.3) it can be seen that *Plectosphaerella cucumerina* and the mycorrhizal fungus *Gigaspora rosae* levels are higher/significantly contributing to the NA fungal community. This is evident because *Plectosphaerella cucumerina* has a significant positive PC1 score (0.36) and has a significant positive PC3 score (0.45). Root pathogen *Colletotrichum coccodes* is significantly contributing to the fungal community in CP samples, deduced by the negative PC3 score 0.66.

Bacterial community

PC1 accounted for 56% of the total variation within CP and NA samples, PC2 accounted for 17% and PC3 accounted for 9% of the fungal relative abundance data set. These first three principle components explained a total variation of 83%, providing good coverage of the data (Table 2.2.5).

Table 2.2.5: Results of principal component analysis of bacterial T-RFLP data from CP treated and NA control crops.

Principal Component	PC1	PC2	PC3
Variation (%)	56.43	17.86	8.55
Cumulative (%)	56.43	74.29	82.84

An Anova of PC scores showed that PC2 was significant between CP and NA samples (P= <*0.05*), however PC1 and PC3 were not significant (P=0.06, P=0.1 respectively).

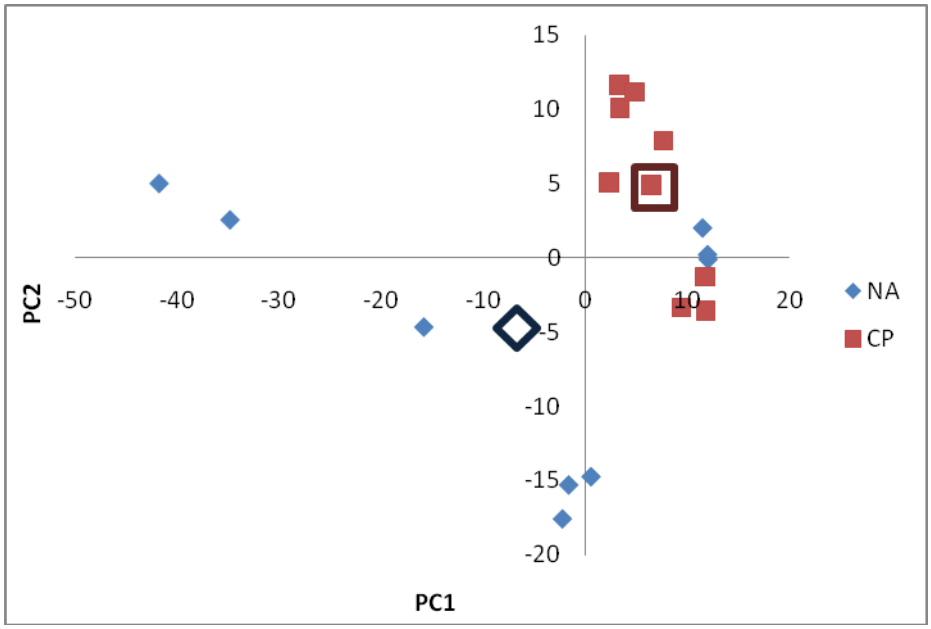

Figure 2.2.4. Ordination plot of PC1 versus PC2 scores using bacterial relative abundance data of Compete™ Plus (CP) treatment and no amendment (NA) control plants.

Note: Large symbols represent mean PC score centroids.

Table 2.2.6: Significant fragment lengths contributing to PC1 and PC2, their possible identity and whether they are associated with CP or NA samples

Fragment Length/Enzyme	PC2	Potential Identity	CP vs. NA
FLH_375	-0.32341	Unknown	NA
FLM_193	-0.26305	Unknown	NA
FLM_371	-0.44014	*Azoarcus sp./Burkholderia phytofirmans/ xenovorans*	NA
FLM_375	-0.35943	Unknown	NA

Plant growth promoting organisms *Azoarcus sp., Burkholderia phytofirmans* and/or *Burkholderia xenovorans* were identified as significantly contributing to the non-amended sample community (Table 2.2.6).

2.3 UV treatment of NFT solution

Fungal root pathogens *Pythium dissocotum* and *Colletotrichum coccodes* were isolated from both UV treated and untreated roots. Previous studies have shown a relative predominance of *P. dissocotum* (or Pythium group F) and a low diversity of *Pythium* spp. in tomatoes grown in soilless culture (Vallance *et al.,* 2010).

The tomato crop grown with UV treated water had significantly higher levels of *Colletotrichum coccodes* than the untreated control crops (P= <0.01) (Figure 2.3.1). T-RFLP did not pick up *Pythium dissocotum*.

Table 2.3.1: Recovery of fungi by isolation from tomato roots in UV and non- UV treated water, their likely identify and sequence homology from PCR amplification

Water	**Sample disinfection**	**Likely organism**	**Homology (%)**
UV treatment	Nil	*Penicillium olsonii*	99
UV	SS	*Plectosphaerella cucumerina*	99
UV	SS	*Colletotrichum coccodes*	98
UV	Nil	*Pythium dissocotum*	97
Nil	Nil	*Colletotrichum coccodes*	99
Nil	SS	*Pythium dissocotum*	98

SS – surface sterilised in sodium hypochlorite.

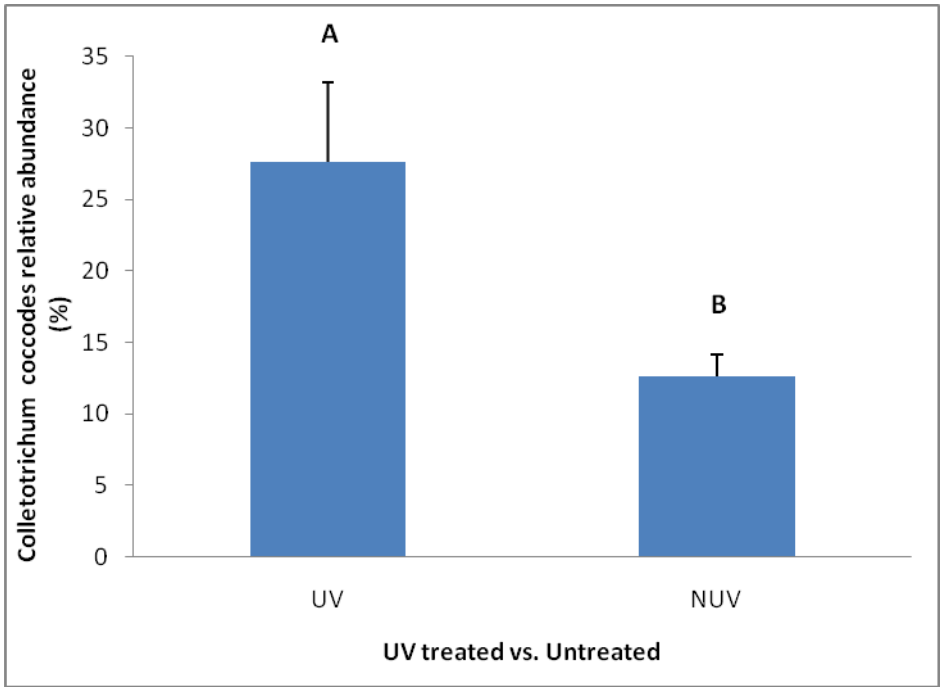

Figure 2.3.1. Mean relative percentage abundance levels of *Colletotrichum coccodes* present on roots from UV treated water and untreated water as determined by T-RFLP output data. Letters in common indicate samples are not significantly different.

T-RFLP analysis

Microbial diversity was not significantly different between UV (UV) and untreated (NUV) samples (P= 0.1 for both bacterial and fungal communities) (Figure 2.3.2).

Fungal species richness was significantly higher in the UV treated crop (P= <0.01), but bacterial diversity did not differ significantly (P= 0.7) (Figure 2.3.3).

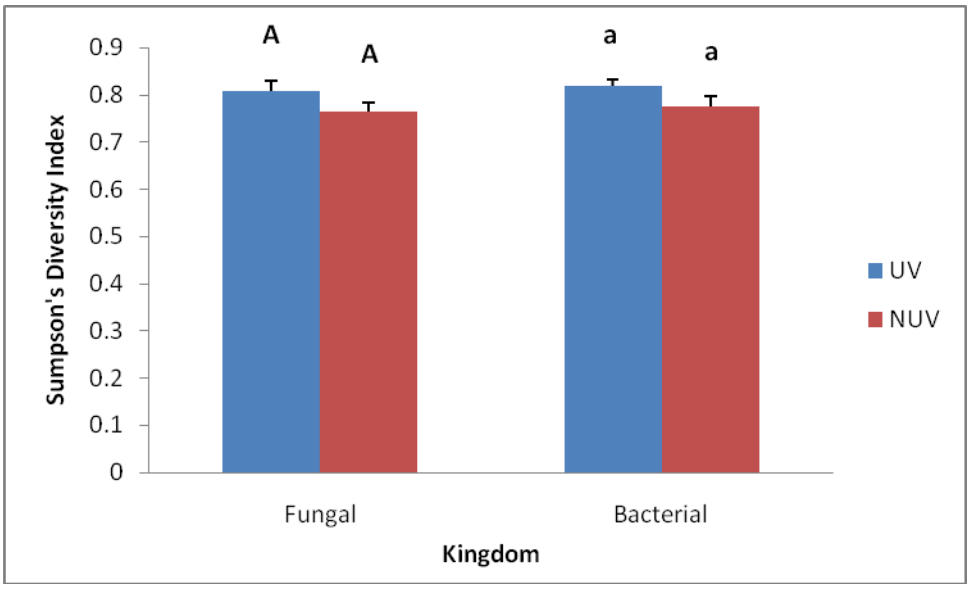

Figure 2.3.2. Effect of UV treatment on microbial diversity calculated using Simpson's Diversity Index. Error bars represent 1 standard error from the mean. Letters in common indicate samples are not significantly different.

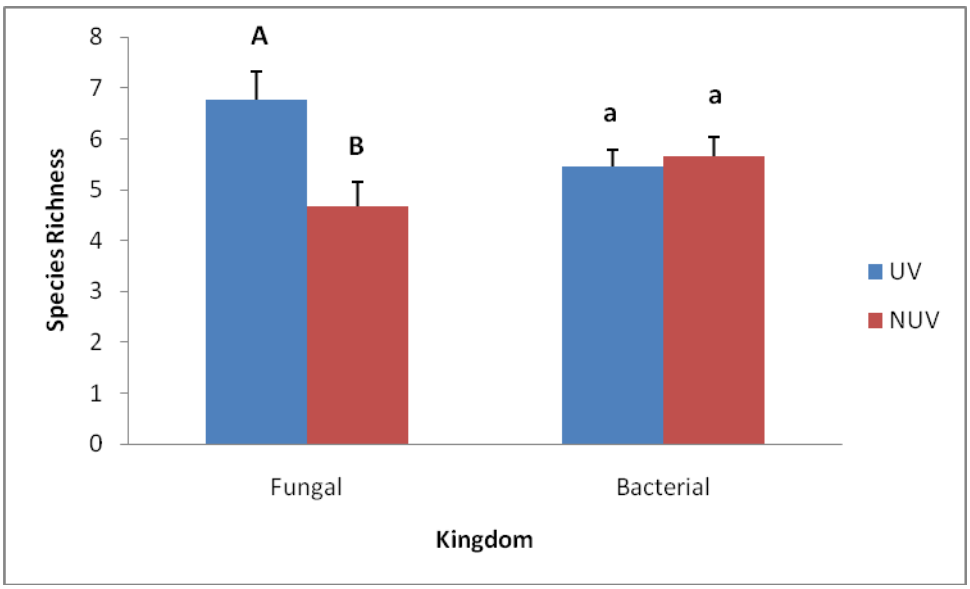

Figure 2.3.3. Effect of UV treatment on microbial diversity calculated using Simpson's Diversity Index. Error bars represent 1 standard error from the mean. Letters in common indicate samples are not significantly different.

Fungal community

In a Principal Component Analysis of the fungal community, principal component 1 (PC1) accounted for 100% of the total variation within UV and non-UV treated samples (Figure 2.3.4). An Anova of PC scores showed that PC1 was not significant between UV and untreated samples (P=0.8). PC analysis found no variation between UV treated and untreated fungal communities.

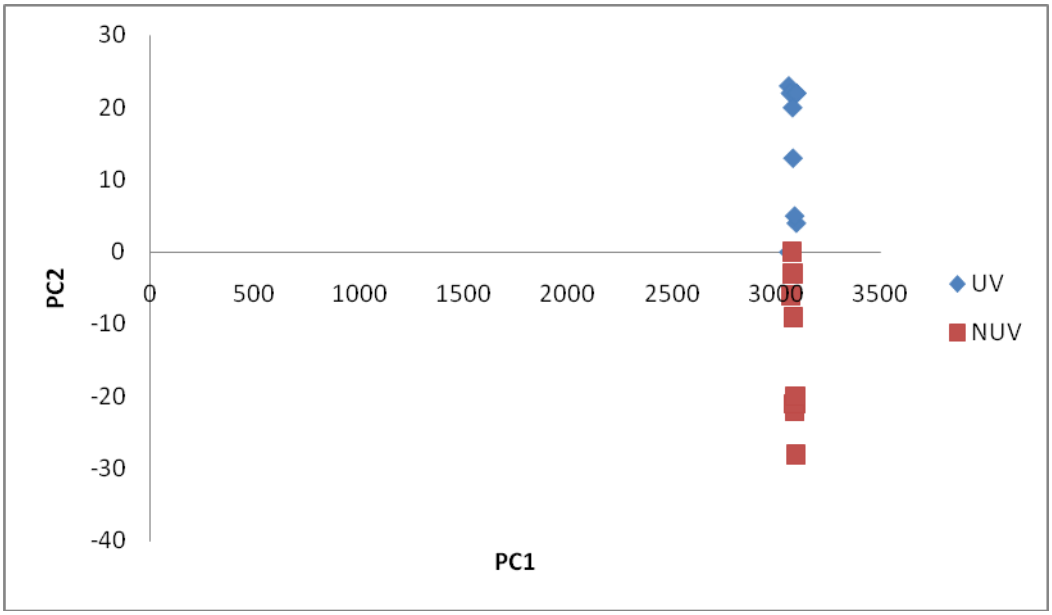

Figure 2.3.4. Ordination plot of PC1 versus PC2 scores using fungal relative abundance data of UV treated (UV) and non- UV treated (NUV) plants.

Bacterial community

Principal component 1 (PC1) accounted for 100% of the total variation within UV and non-UV treated samples.

Results of principal component analysis of bacterial T-RFLP data from UV vs. Non UV treated Crops are shown in Figure 2.3.5.

An Anova of PC scores showed that PC1 was not significant between UV and untreated samples (P= 0.1). PCA analysis found no variation between UV treated and untreated bacterial communities.

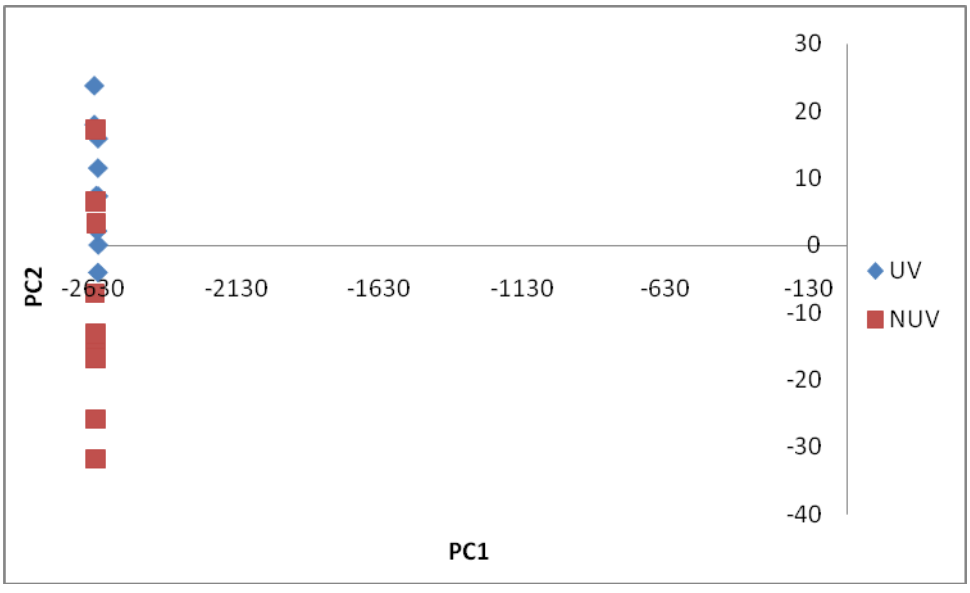

Figure 2.3.5. Ordination plot of PC1 versus PC2 scores using bacterial relative abundance data of UV treated (UV) and non- UV treated (NUV) plants.

Conclusions

Root mat

The results of the sample comparisons indicate that *Agrobacterium radiobacter*, a vector of the Ri plasmid causing root mat disease, was present in roots of both visibly healthy and root mat affected plants. At least two strains were detected, both of which were originally isolated from UK tomatoes with root mat disease. No fungal or bacterial species were identified as being present at more abundant levels in the unaffected plants. Possibly the lack of root mat symptoms in these plants was due to lack of the Ri plasmid; or that plants were infected by rhizogenic *A. radiobacter* but that symptoms had not yet developed.

Compete™ Plus

Our work indicated that Compete™ Plus drenches as used on a woodfibre crop increased bacterial diversity and species richness, but not fungal diversity or species richness. Bacterial species were the main components we identified in the product. There was evidence that Compete ™ Plus may have reduced levels of *P. cucumerina* on roots.

UV treatment of NFT solution

Our work indicated that UV treatment of recycled NFT solution, as used on a particular nursery (nursery 5 for routine monitoring), appeared to increase fungal species richness and levels of *C. coccodes* on tomato roots. The failure of UV treatment to control *C. coccodes* is not unexpected given that dark-coloured fungal structures, such as the pycnidia of this species, are generally resistant to UV light treatment.

UV treatment had no effect on bacterial diversity or species richness. This result was somewhat surprising as bacterial communities are reported as major components of hydroponic systems (Valance *et al*., 2010), and are generally susceptible to UV treatment. Possibly the UV equipment on the nursery had not been functioning correctly in the period prior to collection of root samples.

3. Effect of some soil amendments on tomato root health and plant survival

Introduction

Root disease problems tend to increase with continuous cultivation in soil, particularly if there is no soil disinfestation treatment between crops as is usual in organic cropping. Various soil amendments have been reported to influence occurrence of tomato root disease (see Year 1 and 2 reports). The aim of this experiment was to determine the effect of some soil amendments on tomato root health, plant survival and microbial populations associated with roots.

Materials and methods

Site and crop details

The experiment was done in an organic tomato crop on the Isle of Wight. The site was used for arable cropping prior to organic conversion and erection of the glasshouse. Organic tomatoes have been grown in the house for 5 years. Leaf yellowing, poor growth and plant death have become an increasing problem over successive years, even with plants grown on Beaufort or similar rootstocks with resistance to some pathogens. A range of fungal pathogens had been recovered from affected plants over this period including *Colletotrichum coccodes*, *Fusarium* spp., *Spongospora subterranea*, *Thielaviopsis basicola* and *Verticillium albo-atrum*; symptoms typical of brown and corky root rot (*Pyrenochaeta lycopersici*) were also present. The experiment was located in an area where there was widespread poor growth in 2009.

Soil was amended in winter 2009 prior to planting cv. Piccolo on Beaufort rootstock on 18 February 2010. Plants were planted at 50 cm spacing along a row. Two heads were taken from each plant to give a density of 2/m^2. Other than the treatments detailed below, the crop was grown to commercial standards according to normal practice of the host nursery. Base fertiliser dressing (rooster pellets) was amended to take account of the nitrogen present in Biofence and the nitrogen lock-up following addition of bark.

Treatments

1. Untreated.
2. PHC Compete Plus applied at 0.23 g/pot in alternation with PHC Colonize AG at 0.23 g/pot in 340 ml water/pot at monthly intervals. PHC Compete Plus was also applied in propagation.
3. Trianum-P applied in propagation at 1.5 g/m^2 in 2.5-5 litres water immediately after sowing, and at 15 ml/1000 plants (0.088 ml/L) in 340 ml/pot 1 week after planting and again 1 month later.
4. Composted green waste applied at 25 kg/linear m of bed and incorporated to around 23 cm depth at 1 month before planting.
5. Melcourt Composted Fine Bark-FSC applied at 0.345 m^3/m^2 and incorporated as above (i.e. 1 part bark to 3 parts soil by volume).
6. Biofence pellets applied at 250 g/m^2, incorporated to 23 cm depth as above, watered in and covered with polythene.

Details of the specification of individual products are shown in Table 3.1.

Experimental design and statistical analyses

The experiment was a randomised block design with six fold replication. Individual plots consisted of an island bed of 18 planting pots (36 plants) spaced at 50 cm (plot dimension were 9.5 m x 0.8 m). The six plots in a block were arranged along one row, omitting 10 m near the row ends. The six blocks were arranged in adjacent rows of crop comprising 2 stanchion rows (at the edge) and 4 bay rows. Two heads were taken per plant to give a density of 4/m^2.

Table 3.1: Details of soil amendments – 2010

Product	Specification
PHC Compete Plus	*Bacillus, Pseudomonas, Streptomyces, Trichoderma* formulated with vitamins, humic acids and seaweed extract.
PHC Colonize AG	A plant flavonoid that stimulates mycorrhizal fungi.
Trianum-P	*Trichoderma harzianum* strain T-22, applied once in propagation (December 2009) and twice after planting (February and March 2010)
Composted Green Waste (CGW)	Primarily composted tomato crop waste, produced on site.
Melcourt Composted Fine Bark-FSC	A soil conditioner consisting of matured (at least 12 weeks) British conifer bark with a particle size distribution of 1-10 mm and <5% wood content. Bulk density 390-440 kg/m^3, dry matter 55%, organic matter 85%, pH 4.5-5.5, low in N, P, Mg; medium level K, electrical conductivity 150 µS/cm.
Biofence	Pellets of Caliente mustard seed meal (*Brassica juncea* cv. carinata) a soil fertiliser.

Soil and root microbial assessments

A soil sample was taken before any amendments were made to determine biological activity as measured by T-RFLP and also by conventional tests for micro-organisms (Soil Foodweb Analysis, Laverstoke Park Farm Laboratory Service; www.laverstokepark.co.uk). Root samples were taken on 19 April (8 weeks after planting), 28 May (around peak fruit load) and 26 July (main season) to determine microbial populations by T-RFLP.

Root samples were taken from treatments 1 and 3 to determine the effect of Trianum-P treatment on establishment of *Trichoderma* spp. Samples were taken on 25 February 2010, 1 week after planting (to determine effect of propagation treatment), and on 6 April 2010, 2 weeks after a second application of Trianum-P on the nursery (to determine effect of production nursery treatments). On 25 February, samples were taken from the side at the bottom half of the propagation cube and from the soil. On 6 April, young roots were taken from the soil. Samples were posted to Koppert BV in the Netherlands for determination of *Trichoderma* populations as number of colony forming units per gram of dried root (cfu/g). Sub-samples were tested to determine if the strain cultured from roots was identical to the strain present in Trianum-P (i.e. T-22).

Bulk samples of soil from three replicates of each treatment were taken for Soil Foodweb Analysis at the end of cropping. A soil sample from the whole trial area was taken at the end of cropping and tested for *Verticillium dahliae* by ADAS High Mowthorpe (Harris & Yang, 1997), for *V. dahliae* and *V. albo-atrum* by PCR molecular tests at Fera and by T-RFLP at Nottingham University. A sample of roots showing different symptoms was collected at the final assessment and tested for possible causal fungi by isolation onto agar at ADAS Boxworth.

Crop assessments

Plants were assessed at intervals to determine the number of wilting, yellowing and dead heads. At the end of cropping, the number of green stem bases and of live heads remaining was assessed. Twenty plants in each plot were examined for vascular staining in the stem base. These plants were also forked up in the first three replicates, and the extent and health of roots were estimated. Fruit yield was not recorded. Statistical analysis was by generalised linear models or ANOVA in Genstat.

Results and discussion

Leaf symptoms and plant death

At the first assessment on 25 May there was significantly less leaf necrosis in the plant head where composted green waste was used (Table 3.2). Possibly this was due to greater moisture retention and availability at times of temperature stress. Leaf yellowing occurred at a low level in this treatment and was absent in most other treatments. The incidence of wilting or dead heads was low with no significant differences between treatments.

Table 3.2: Effect of soil amendments on appearance of soil-grown tomato, cv. Piccolo on Beaufort rootstock – 25 May 2010

Treatment	Mean % heads affected by		Wilted or dead
	Leaf necrosis (top leaves)	Leaf yellowing	
1. Untreated	59.1 (5.5)	0	0.5 (0.3)
2. Compete Plus	51.5 (5.6)	0	1.4 (0.5)
3. Trianum	60.2 (5.5)	0	0 (0)
4. CGW	25.0 (4.9)	9.0 (0.7)	0.9 (0.4)
5. Melcourt bark	51.1 (5.6)	0.4 (0.1)	0.5 (0.3)
6. Biofence	63.7 (5.4)	0	0.5 (0.3)
Significance (25 df)	<0.001	<0.001	NS

NS - not significant; ( ) – standard error.

At an assessment on 14 July, the mean number of live heads/plot (assessed on one side of the row) ranged from 26.7 to 27.8 with no significant differences between treatments.

At the final assessment on 15 October, there was no difference between treatments in the % green stem bases, and no reduction in the occurrence of vascular staining (Table 3.3). At this stage, the number of live heads per plot was slightly lower in the CGW plots (46/plot) than in the untreated (53/plot). It is possible that this difference may have resulted from broken stems rather than root disease.

Table 3.3: Effect of soil amendments on plant survival and stem base vascular staining in tomato cv. Piccolo on Beaufort rootstock – 15 October 2010

Treatment	Mean % green stem bases	Mean no. live heads/plot	Mean % green stem bases with vascular staining
1. Untreated	94 (1.7)	53	13 (3.3)
2. Compete Plus	91 (2.0)	51	19 (3.8)
3. Trianum	94 (1.7)	52	19 (3.8)
4. CGW	90 (2.1)	46	23 (4.0)
5. Melcourt bark	94 (1.8)	50	33 (4.5)
6. Biofence	91 (2.1)	48	15 (3.4)
Significance (25 df)	NS	0.027	0.023
LSD	-	3.9	-

Root symptoms

There were no significant differences between treatments in root extent or root length affected by black dot or corkiness (Table 3.4).

Isolation tests on root samples collected at the final assessment confirmed *C. coccodes* was associated with the black dot symptom. A *Fusarium* sp., probably *F. oxysporum*, was isolated quite consistently from the corkiness symptom.

Table 3.4: Effect of soil amendments on extent and appearance of roots of tomato cv. Piccolo on Beaufort rootstock – 5 November 2010

Treatment	Root extent (0 – 5)	Mean % root length affected by		
		Corkiness	Black dot	Corkiness + black dot
1. Untreated	2.9	5.2	11.8	17.0
2. Compete Plus	2.6	9.0	12.5	21.5
3. Trianum P	2.8	4.4	10.6	15.1
4. CGW	2.4	8.6	20.2	28.8
5. Melcourt bark	2.5	5.5	11.4	16.9
6. Biofence	2.8	3.6	29.3	22.9
Significance (25 df)	NS	NS	NS	NS
LSD	0.47	5.53	12.2	10.59

Root extent – 0 = no roots, 5 = vigorous root system

Soil chemistry

The effect of CGW, bark and Biofence on soil chemical properties is shown in Table 3.5. The CGW treatment increased soil pH, conductivity and levels of chloride, potassium, sodium, nitrate, sulphate and boron. Bark increased levels of potassium, magnesium, calcium, and iron and decreased ammonium. These changes are consistent with previous results reported by growers when using such amendments. Biofence had no effect on soil chemical properties.

Table 3.5. Effect of organic amendments on soil chemical properties, tomato root health trial, Isle of Wight – July 2010

Determinand	Untreated (T1)	CGW (T4)	Bark (T5)	Biofence (T6)
pH	6.61	**7.60**	6.63	6.84
Density (kg/m^3)	986	930	881	911
Dry matter (%)	59.1	54.8	57.2	62.1
Dry density (kg/m^3)	582.7	509.6	503.9	565.7
Chloride (mg/l)	145.0	**521.9**	126.6	118.2
Phosphorus (mg/l)	47.8	33.9	49.4	43.8
Potassium (mg/l)	349.1	**1521.8**	**151.5**	214.4
Magnesium (mg/l)	35.2	34.5	**40.7**	29.7
Calcium (mg/l)	177.6	169.5	**199.2**	181.2
Sodium (mg/l)	143.5	**207.2**	148.8	125.0
Conductivity (uS/cm)	526	**1140**	413	421
Ammonia-N (mg/l)	52.2	45.1	**33.0**	38.6
Nitrate-N (mg/l)	155.2	**258.1**	127.7	127.0
Total Soluble N (mg/l)	207.4	303.2	160.7	165.6
Sulphate (mg/l)	305.3	**626.8**	272.0	245.1
Boron (mg/l)	0.76	**1.07**	0.51	0.39
Copper (mg/l)	0.15	0.19	0.19	0.14
Manganese (mg/l)	0.10	0.19	0.55	0.07
Zinc (mg/l)	0.18	0.19	0.46	0.12
Iron (mg/l)	4.89	12.19	**42.53**	5.78

Values greatly different from those of untreated soil are shown in bold.

Soil Foodweb analysis

The Soil Foodweb analysis (Table 3.6) showed that in untreated soil, levels of bacteria, fungi and protozoa were all greater after cropping (October) than pre-planting (February). Active bacteria appeared to be reduced by Biofence and not by other treatments. Active fungi appeared to be reduced by the composted green waste, bark and Biofence treatments. Trianum P appeared to increase the levels of active fungi.

Flagellate and ciliate protozoa appeared to be reduced by all treatments; amoebic protozoa were at low levels in all treatments. Nematode levels (not shown) were low at both sample dates (0.1 - 0.4 nematodes/g soil) and in all treatments.

The Soil FoodWeb interpretation is that active bacteria are high in all treatments, active fungi are high in T1-T3 (Untreated, Compete^{TM} Plus and Trianum), and ciliate protozoa are

high in all treatments; total fungi and amoebic protozoa and total nematodes were low in all treatments (Table 3.6). Various ratios as used by the Soil FoodWeb analysis are summarised in Table 3.7.

Table 3.6: Effect of pre-plant soil amendments and post-plant microbial drenches to tomato on soil biological activity as determined by a Soil FoodWeb analysis[a]

Treatment	Bacterial (µg/g)		Fungi (µg/g)		Protozoa (numbers/g)		
	Active	Total	Active	Total	Flagellate (x 10^3)	Amoebic (x 10^3)	Ciliate (x $10^{3)}$
Baseline (Feb 2010)							
Untreated	8	204	<1	4	3	47	<1
End of cropping (Oct 2010)							
1. Untreated	78	269	33	48	95	0	30
2. Compete Plus	70	135	17	31	30	<1	4
3. Trianum P	73	186	59	74	36	1	<1
4. CGW	89	132	4	49	13	<1	2
5. Bark	72	250	8	23	12	1	<1
6. Biofence	47	138	4	15	9	1	<1
Expected range Low	10	150	10	150	10	10	0.05
High	25	300	25	300	-	-	0.1

[a] Bulk soil samples, 0-20 cm, from 3 replicates; mean of 3 Foodweb analyses/sample; values expressed per g soil fresh weight.

Table 3.7: Soil FoodWeb interpretation of fungal/bacterial ratios recorded in three tests on each sample from soil amendments trial – 2010

Treatment	Total fungi/ total bacteria	Active fungi/ total fungi	Active bacteria/ total bacteria	Active fungi/ active bacteria
Baseline (Feb)				
Untreated	LLL	LGL	LLL	LLL
End of cropping (Oct)				
1. Untreated	LLL	HHH	HHH	GLL
2. Compete Plus	LLL	HHH	HHH	LLL
3. Trianum P	LLL	HHH	HHH	GGL
4. CGW	LLL	GLL	HHH	LLL
5. Bark	LLL	HHH	HHH	LLL
6. Biofence	LLL	HLH	HHH	LLL

L – low, H – high, G – good; three repeat tests were done on each soil.

The total fungi/total bacteria ratio was low in all treatments. A low value indicates the soil is bacterial dominated. It is reported in the Soil FoodWeb report that such soils will lack disease suppression, nutrient retention and ability to build up soil structure.

The active fungi/total fungi ratio was generally high. A high value indicates fungi are growing and should result in an increase in total fungal biomass; a low value indicates low activity.

The active bacteria/total bacteria ratio was high during cropping in all treatments. A high value indicates good bacterial activity; a low value, as found pre-planting, indicates low activity.

The active fungi/active bacteria ratio was low or good. A low value indicates the soil is becoming more bacterial.

The Soil Foodweb analysis reports do not currently provide interpretation of results in terms of root disease risk in a tomato crop. Our work shows that even when there is little visible root disease in a crop, differences in Soil FoodWeb reported values can occur. Development of further guidance on interpretation of Soil FoodWeb values would be useful.

Effect on Trianum-P treatment on soil *Trichoderma* populations

Interpretation of relative levels of *Trichoderma* species associated with root and soil samples determined by Koppert BV was as shown below:

Relative level	Density of *Trichoderma* sp (cfu/g)	
0	0 - 1×10^3	Not present or trace
1	1×10^3 - 1×10^4	Moderately abundant
2	1×10^4 - 1×10^5	Abundant
3	> 1×10^5	Very abundant

Although *Trichoderma* spp. were detected at high levels on root samples collected on 25 February, there was no consistent difference in levels between plants treated with Trianum-P during propagation of the tomato plants, and untreated plants (Table 3.8). None of the isolates examined were identified as T-22. This result indicates that there was a high level of wild type *Trichoderma* spp. that established on roots either during propagation or within a few days of planting.

On the root samples collected on 6 April, *Trichoderma* spp. were detected at high levels on plants treated with Trianum-P and were barely present or moderately abundant on plants not treated (Table 3.8). The level of *Trichoderma* spp. in untreated soil from the edge of a bed was also very low. This result indicates that application of Trianum-P after planting influences the level of *Trichoderma* spp. in the rhizosphere.

Table 3.8: Detection of *Trichoderma* species on roots of soil-grown tomato plants treated and untreated with Trianum-P (*Trichoderma harzianum* T-22), Isle of Wight – 2010

Sample	Density of *Trichoderma spp*. as cfu/g (index) on samples collected:	
	25 February	6 April
1. Untreated, plot 3	6.1×10^5 (3)	9.1×10^4 (1)
2. Untreated, plot 23	4.3×10^5 (3)	6.1×10^3 (1)
3. Trianum-P treated, plot 5	5.2×10^5 (3)	9.0×10^4 (2)
4. Trianum-P treated, plot 19/26	1.8×10^4 (2)	1.5×10^5 (3)
5. Soil from untreated, plot 15	NT -	1.5×10^3 (1)

NT – not tested

Potential pathogens present in the soil

Tests on a soil sample from the overall trial area for *V. dahliae* by the agar plate method indicated a low level of this fungus (0.1 cfu/g). A QPCR test on the same soil sample for *V. dahliae* and *V. albo-atrum* at Fera did not detect either fungus (<250 fg/g soil).

T-RFLP analysis of soil

Likely fungi present in soil as determined by T-RFLP, from soil sampled from across all treatments, are listed in Table 3.9. The likely pathogens *Botrytis cinerea, Colletotrichum sp., Fusarium oxysporum* and *Rhizoctonia solani* were detected pre-planting. The likely pathogens *Colletotrichum coccodes, Macrophomina phaseolina* and *Pyrenochaeta lycopersici* were detected at the end of the trial. Interestingly, *Plectosphaerella cucumerina*, commonly detected on tomato roots during this project, was not detected in the soil. Similarly, neither *V. dahliae* (detected by an isolation test) nor other *Verticillium* species were detected. These results may indicate differential recovery of DNA from soil than from roots.

Table 3.9: Occurrence of likely fungal pathogens and saprophytes in soil from the trial site as determined by T-RFLP

Likely fungus	Terminal Restriction Fragment (TRF) length	Minimum relative amount (%)	
		Pre-planting	End of trial
Pathogens			
Botrytis cinerea	175	2.63	0
Colletotrichum sp.	329	0.29	3.47
Fusarium oxysporum	120	5.06	0
Macrophomina phaseolina	188	0	1.80
Pyrenochaeta lycopersici	189	0	1.80
Rhizoctonia solani	175	1.03	0
Saprophytes			
Aspergillus sydowii	339	1.06	10.93
Aspergillus ustus	341	1.06	10.93
Cladosporium herbarum	322	6.44	1.81
Epicoccum sp.	190	0	1.80
Gigaspora rosae	342	2.42	14.53
Penicillium purpurogenum	329	1.91	1.91

T-RFLP analysis of roots

When examined for relative abundance of different micro-organisms, it was found that soil amendment had no significant effect (P = 0.939). Fungal species that made a significant contribution to the rhizosphere populations were: *Aspergillus* sp., *Botrytis* sp., *Chaetomium* sp., *Gigaspora* sp., *Glomus* sp., *Leptosphaeria* sp., *Penicillium* sp., *Phytophthora* sp., *Pythium* sp. and *Verticillium* spp.

There were no differences between treatments in fungal diversity calculated using Simpson's Diversity Index (Figure 3.1) or microbial species richness (Figure 3.3).

Fungal diversity over all treatments was greatest at the first sampling (0.60) and significantly lower (P <0.01) at the third samplings (0.40) whereas bacterial diversity was unaffected by sampling time (Figure 3.2). Fungal species richness decreased with time while bacterial species richness increased (Figure 3.4).

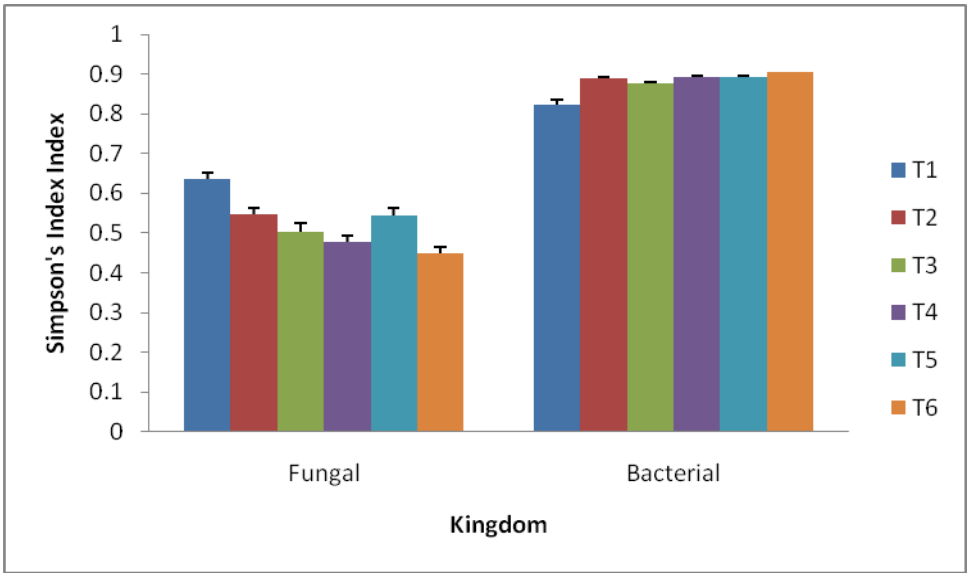

Figure 3.1. The effect of soil amendment on microbial diversity calculated using the Simpson's Diversity Index. Error bars indicate one standard error (T1=Untreated, T2=Compete Plus/Colonize, T3=Trianum, T4=WSG Green Waste, T5=Composted Fine Bark, T6=Biofence).

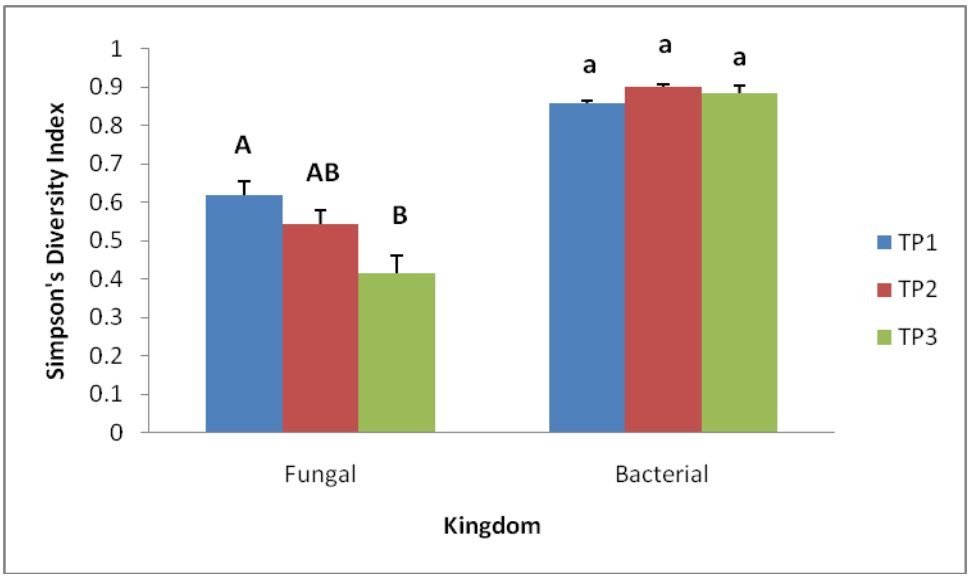

Figure 3.2. The effect of time on microbial diversity calculated using the Simpson's Diversity Index. Error bars indicate one standard error. Letters in common are not significantly different (TP1 = 1 week after planting; TP2 = first fruit; TP3 = mid August).

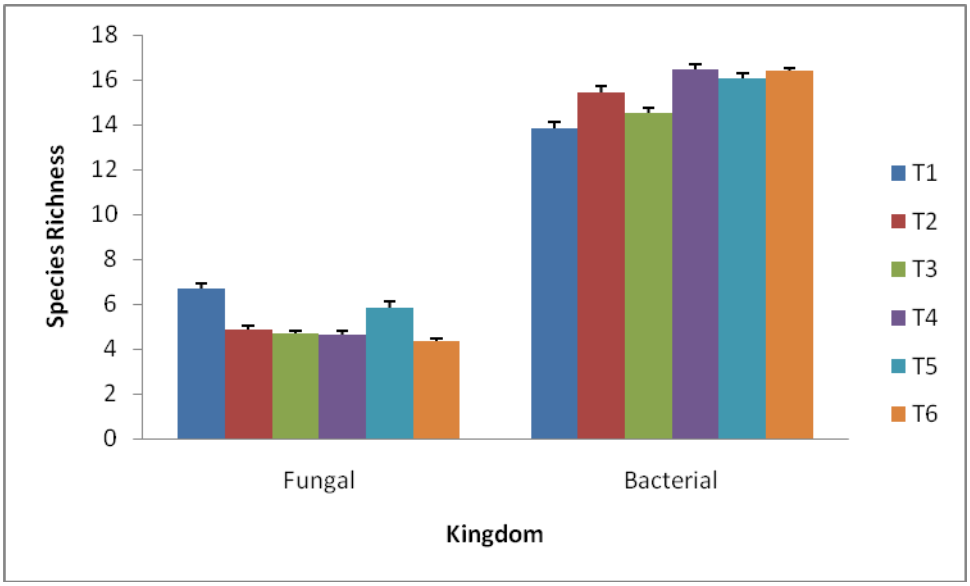

Figure 3.3. The effect of soil amendment on microbial species richness. Error bars indicate one standard error (T1=Untreated, T2=Compete Plus/Colonize, T3=Trianum, T4=WSG Green Waste, T5=Composted Fine Bark, T6=Biofence).

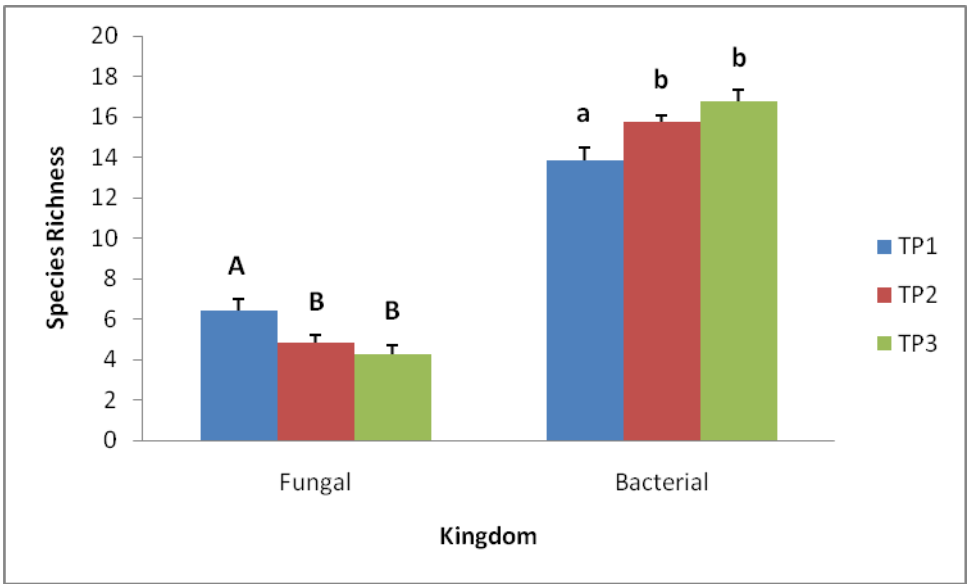

Figure 3.4. The effect of time/plant age on microbial species richness. Error bars indicate one standard error. Letters in common are not significantly different.

The microbial communities were examined by PCA.

Fungal community

The first three principal components accounted for 70% of variation (Table 3.10). PC1 was significant between sampling times (P= <0.01) but not soil treatment (P = 0.09). PC2 was significant between sampling times and treatments (P= <0.01 for both) (Figures 3.5-3.6). PC3 was not significant.

The possible identity of significant fragment lengths contributing to PC1 were *Cladosporium* sp. and *Colletotrichum coccodes*, which were associated with the final sampling (TP3).

Table 3.10: Results of principal component analysis of fungal T-RFLP data from soil amendment trial

Principal Component	PC1	PC2	PC3
Variation (%)	34.84	26.84	8.57
Cumulative (%)	34.84	61.68	70.25

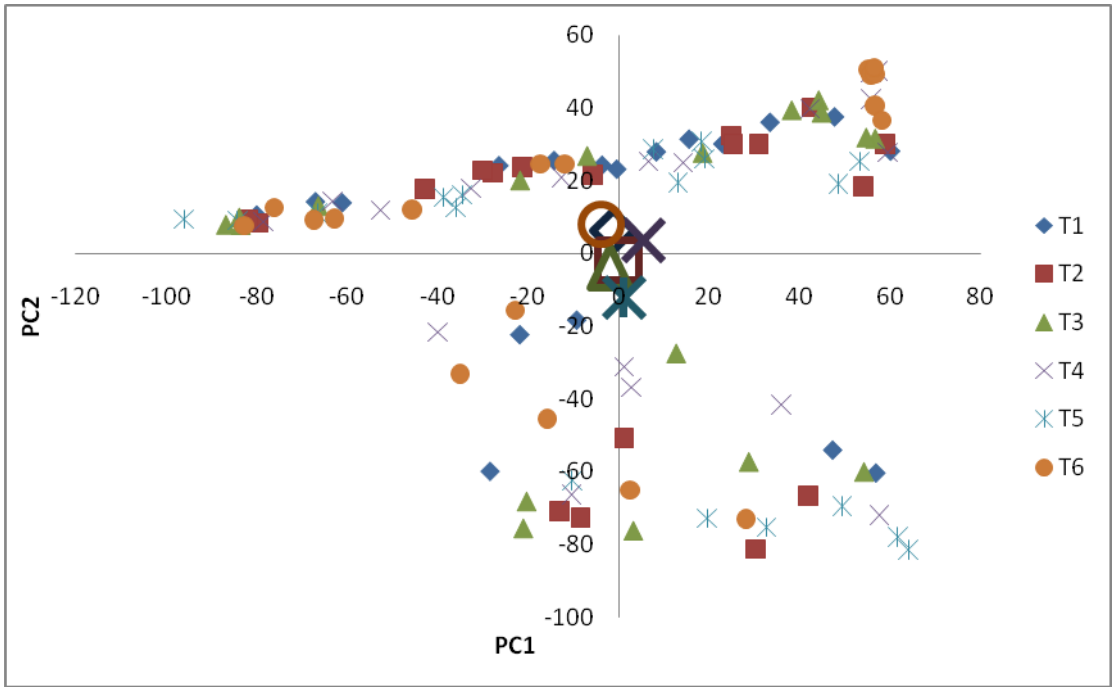

Figure 3.5. Ordination plot of PC1 versus PC2 scores for fungal relative abundance data from soil amendment trial (T1=Untreated, T2=Compete Plus/Colonize, T3=Trianum, T4=WSG Green Waste, T5=Composted Fine Bark, T6=Biofence).

Note: Large symbols represent mean PC score centroids.

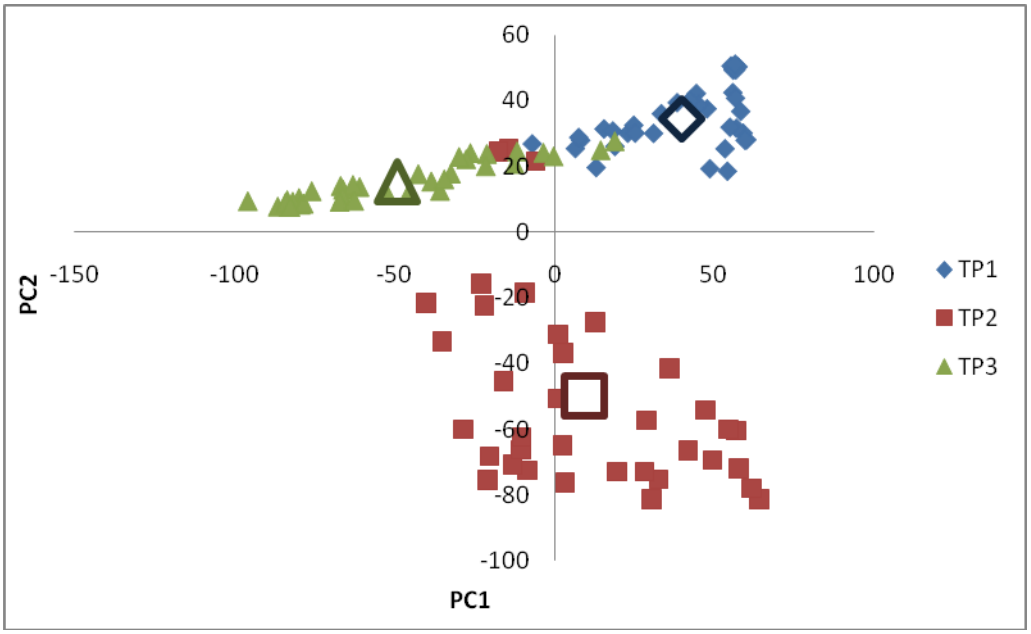

Figure 3.6. Ordination plot of PC1 versus PC2 scores for fungal relative abundance data from soil amendment trial (TP1 = early season, TP2 = mid season, TP3 = late season)

Note: Large symbols represent mean PC score centroids.

Bacterial community

The first three principal components accounted for 62% of variation. PC1 was significant between sampling times (P= <0.01) but not treatments (P = 0.06). PC2 was significant for separating both time points (P= <0.01) and treatments (P= <0.05). PC3 was not significant. Ordination plots of the effect of treatments (Figure 3.7) and sampling time (Figure 3.8) are shown below.

The possible identity of a significant fragment length contributing to treatment differences was *Clostridium phytofermentans* (T1/T3/T4/T5/T6). The possible identity of significant fragment lengths contributing to sampling time differences were: *Methylobacterium/ Oligotropha carboxidovorans/Agrobacterium radiobacter* (TP2/TP3) and *Clostridium phytofermentans* (TP1).

Table 3.11: Results of principal component analysis of fungal T-RFLP data from soil amendment trial

Principal Component	PC1	PC2	PC3
Variation (%)	27.47	25.67	9.09
Cumulative (%)	27.47	53.14	62.23

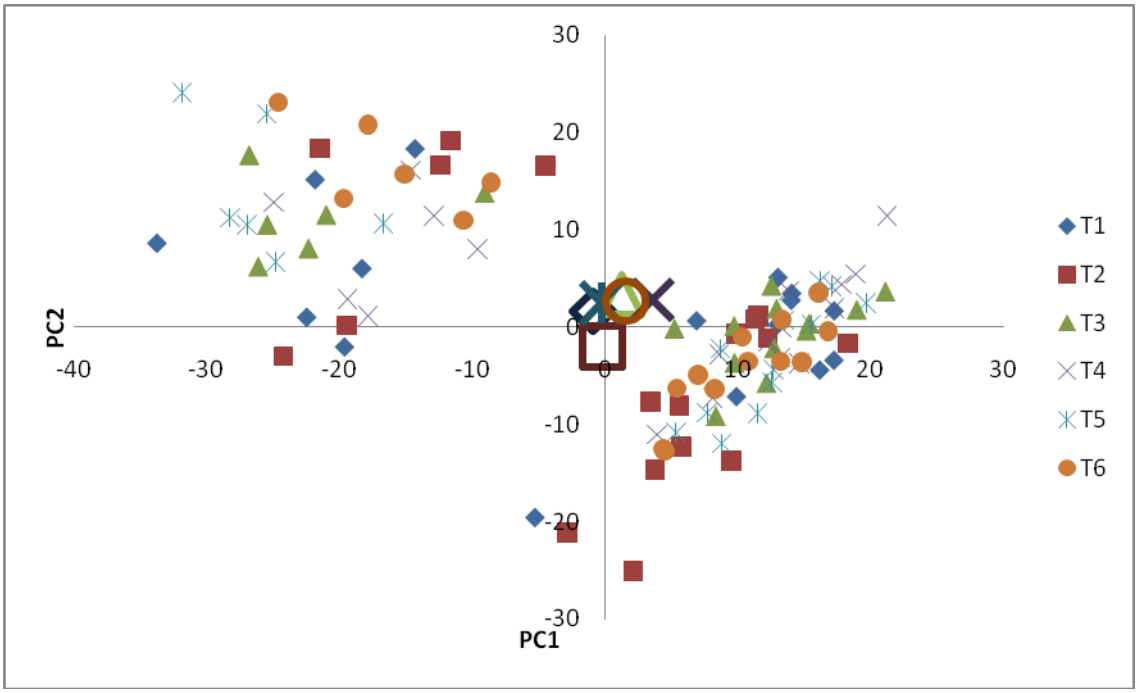

Figure 3.7. Ordination plot of PC1 versus PC2 scores for bacterial relative abundance data from soil amendment trial (T1=Untreated, T2=Compete Plus/Colonize, T3=Trianum, T4=WSG Green Waste, T5=Composted Fine Bark, T6=Biofence).

Note: Large symbols represent mean PC score centroids.

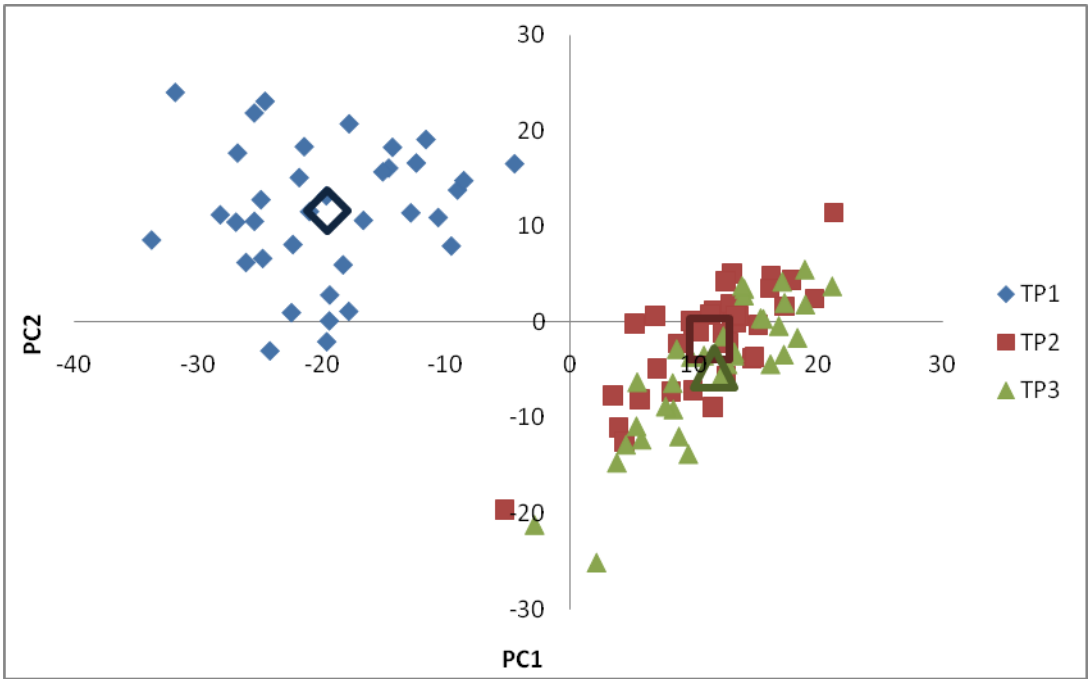

Figure 3.8. Ordination plot of PC1 versus PC2 scores for bacterial relative abundance data from soil amendment trial (TP1 = early season, TP2 = mid season, TP3 = late season).
Note: Large symbols represent mean PC score centroids.

Likely fungal pathogens identified by FRAGSORT included *Botrytis cinerea, Collectotrichum coccodes, Macrophomina phaseolina, Plectosphaerella cucumerina, Pyrenochaeta lycopersici* and *Verticillium nigrescens*. The relative abundance of these selected fungi according to treatment and sampling time is shown in Table 3.12 and 3.13; a full listing is given in Appendix 2.

Table 3.12: Occurrence and relative abundance (%) of selected fungal pathogens associated with different soil treatments

Likely fungal Pathogens	TRF (Hae III)	T1 Unt	T2 CP	T3 Tri	T4 CGW	T5 Bark	T6 BioF
Botrytis cinerea	319	-	-	-	1.3	2.2	0.7
C. coccodes	153	36.6	40.9	43.5	33.5	39.1	45.4
M. phaseolina	327	9.8	8.0	7.9	0.7	2.0	2.6
P. cucumerina	138	10.8	6.7	8.7	3.3	1.1	0.9
Py. lycopersici	328	9.8	8.0	7.9	0.8	2.0	2.6
V. nigrescens		-	-	-	5.9	1.3	1.5

- not detected; Unt – untreated, CP – Compete Plus; Tri – Trianum P; CGW – composted green waste; BioF – Biofence.

Table 3.13: Occurrence and relative abundance (%) of selected fungal pathogens associated with roots of soil grown tomato according to sample time (crop age)

Likely fungal pathogen	TRF (Hae III)	TP1 (after planting)	TP2 (first pick)	TP3 (July)
Botrytis cinerea	319	1.9	1.1	0.0
C. coccodes	153	7.8	3.4	67.1
M. phaseolina	327	5.8	3.8	6.4
P. cucumerina	138	8.0	2.8	4.9
Py. lycopersici	328	5.8	3.4	6.4
V. nigrescens	344	0.0	0.0	2.0

There appeared to be treatment and crop age effects worth further investigation, notably:

- Occurrence of *B. cinerea* with CGW, bark and Biofence;
- Low levels of *M. phaseolina, P. cucumerina* and *P. lycopersici* with CGW, bark and Biofence;
- Occurrence of *V. nigrescens* with CGW;
- Large increase in *C. coccodes* at the July sampling.

Possible fungal antagonists identified by FRAGSORT included *Aspergillus* spp., *Gliocladium roseum* and *Penicillium* spp; possible mycorrhizae included *Gigaspora* spp. and *Glomus* spp. The relative abundance of these fungi according to treatment and sampling time are shown in Tables 3.13 and 3.14; a full list is given in Appendix 2.

Table 3.13: Occurrence and relative abundance (%) of selected potential fungal antagonists and mycorrhizae associated with roots of soil grown tomato according to soil treatment

Likely identification	TRF (Hae III)	T1 Unt	T2 CP	T3 Tri	T4 CGW	T5 Bark	T6 BioF
Aspergillus sp.	83/85	-	0.4	-	5.9	1.3	1.5
Gigaspora rosae	342	1.9	1.5	1.6	0.7	0.2	0.5
Gigaspora sp.	348	-	-	2.3	1.8	0.5	0.6
Gliocladium roseum	119	0.3	-	1.7	0.5	0.8	-
Glomus intraradices	378	1.7	-	0.3	-	0.3	-
Glomus mossae	396	0.2	-	-	-	-	0.6
Penicillium variable	71	-	-	1.5	6.5	2.1	2.0

- not detected; Unt – untreated, CP – Compete Plus; Tri – Trianum P; CGW – composted green waste; BioF – Biofence.

Table 3.14: Occurrence and relative abundance (%) of potential fungal antagonists and mycorrhizae associated with soil grown according to sample time (crop age)

Likely identification	TRF (Hae III)	TP1 (after planting)	TP2 (first pick)	TP3 (August)
Aspergillus sp.	83/85	0.6	1.9	2.0
Gigaspora rosae	312	1.6	0.9	0.7
Gigaspora sp.	348	-	-	2.7
Gliocladium roseum	119	0.5	-	1.6
Glomus intraradices	378	1.2	-	-
Glomus mossae	396	-	-	0.5
Penicillium variable	71	-	-	1.9

There appeared to be some possible treatment and crop age effects worth further investigation, notably:

- Association of *Aspergillus* and *Penicillium* spp. with CGW
- Increase of *Aspergillus* and *Penicillium* spp. with crop age

General discussion

It was disappointing in this study that higher levels of root disease did not occur as the trial was located in a house with a long history of root disease problems, notably Verticillium wilt. Possibly an area was chosen that by chance had low levels of pathogens in the soil and/or the crop was grown in a manner unfavourable to root disease. However, T-RFLP tests on roots indicated presence of *Pythium, Phytophthora* and *Verticillium* species, while isolation tests showed the presence of *Colletotrichum coccodes* and a soil test confirmed the presence of *V. dahliae*. A different crop manager in 2010 grew the crop on a much drier regime than in previous years, suggesting this is likely to be a key part of the explanation.

Nevertheless, some useful results were obtained. As in the routine crop monitoring work (Section 1 of this report) it was shown that, for soil-grown crops, fungal diversity on roots decreased with time (crop age). This is probably due to the high pH of the soil (pH 6.6), a level which is sub-optimal for growth of most fungi. Soil pH is a factor likely to exert its influence gradually over time following planting into soil where roots would initially be at a lower pH (around 5.5) in the peat propagation cube. The Soil FoodWeb test supported the T-RFLP results, reporting that the ratios of total fungi to total bacteria, and active fungi to active bacteria, were both low.

Although T-RFLP analysis indicated none of the applied treatments significantly affected microbial diversity on roots, there was some evidence from the Soil FoodWeb test that drenches of Trianum-P increased the ratio of active fungi to active bacteria on roots. However, *Trichoderma* sp. was not detected by T-RFLP in any of the treatments (Appendix 2), so possibly the increase in the ratio of active fungi to bacteria in T3 (Trianum-P drenches) may have been due to an increase of different micro-organism(s). Possibly the failure of T-RFLP to detect a change in fungal diversity may be due to the test detecting total (including non-active) fungi in larger quantities which swamped an effect on active fungi.

T-RFLP indicated that the principal components of the microbial community that contribute to variation over time were likely due to *C. coccodes* and a *Cladosporium* sp. There was a very large increase in the relative abundance of *C. coccodes* on roots between first pick and July. This fungus is reported to be more common in weakened plants, and was confirmed by observation and isolation tests to be at high levels on roots at the end of the trial. Given the large increase of *C. coccodes* on roots mid-season, this pathogen may be more

damaging to yield of tomato than is considered at present. There is evidence from Italy of increasing problems with black dot root rot in soil-grown tomato (L Gullino, University of Turin, pers. comm.). *Cladosporium* sp. is generally a saprophytic genus and might be expected to increase with root age and an increased release of nutrients from damaged/decaying roots.

T-RFLP indicated occurrence of *B. cinerea* and *V. nigrescens* on roots of plants from T4 (composted tomato waste). The potential for composted green waste to introduce pathogens to a crop, or encourage their development, should not be overlooked.

The Soil FoodWeb analysis produced data on relative occurrence of different micro-organisms, including protozoa, in the soil. However, at present there is little guidance available on how to interpret these results in terms of disease risk in a tomato crop. Possibly over time such data could be useful to help understand changes in soil microbial communities. Results on levels of specific individual pathogens, or a range of pathogens and antagonists, as should be possible with a microarray test, are more likely to be useful to tomato growers in the first instance.

T-RFLP indicated that levels of *Aspergillus* spp., *Penicillium* spp. and *Gigaspora* sp. on tomato roots increased with time. These are all potential antagonists to development of some fungal pathogens. The effect of *Aspergillus, Penicillium* and *Gigaspora* on development of important root pathogens under controlled conditions warrants investigation to help determine their potential for disease control. Composted green waste (T4) increased levels of *Aspergillus* and *Penicillium* spp. compared with untreated soil, and may have benefit as a treatment to reduce disease risk in soil-grown crops; there was evidence from T-RFLP tests of reduced levels of *M. phaseolina, P. cucumerina* and *P. lycopersici* in soil amended with composted green waste, compared with untreated soil. However, it cannot be assumed that there is a causal association between increased levels of *Aspergillus* and *Penicillium* and reduced levels of the above-mentioned pathogens.

Previous work has found that the effect of a microbial amendment on roots on disease is likely to vary with the tomato variety. For example, it was found that strains of *Trichoderma atroviride* and *T. harzianum* enhanced growth and systemic resistance against *Botrytis cinerea* in some but not all tomato lines tested (Tucci *et al*., 2011). In our work we tested

soil amendments on the rootstock Beaufort. We would likely have observed greater levels of root disease, and hence better opportunity to determine if soil amendments affected root disease, if we had used an ungrafted variety. However, this is not good commercial practice when growing tomato in soil, especially where there is repeated cropping on a site and the ground is not disinfested between crops.

4. Effect of rootstock variety on tomato root health and plant survival

Introduction

Rootstocks are used to increase plant vigour and to reduce the effects of root disease and nematodes. The aim of this experiment was to compare the effect of six rootstocks on tomato root health, plant survival and microbial populations associated with tomato roots.

Materials and methods

Site and crop details

The experiment was done in an organic tomato crop cv. Roterno on the Isle of Wight. Organic tomatoes had been grown in the house for at least 10 years. The experiment was located in an area where leaf yellowing and poor growth occurred in 2009. The crop was grown according to normal nursery practice. This included incorporation of green waste compost prior to planting and monthly drench treatment with PHC Compete Plus and Colonise AG in alternation. The crop was planted on 29 December 2009.

Treatments		Resistances
1.	Beaufort (De Ruiter)	HR : ToMV/Fol:0,1/For/PI/Va/Vd/Ma/Mi/Mj
2.	Efialto (Enza Zaden)	HR : ToMV/Ff:1-5/Va/Vd/Fol:0,1/For IR : Ma/Mi/Mj
3.	Emperador (Rijks Zwaan)	HR : ToMV/Fol:0,1/For/PI/Va/Vd/Mi/Mj/Ma
4.	Optifort (De Ruiter)	ToMV/Fol:0,1/For/Pl/Va/Vd/Ma/Mi/Mj
5.	Stallone (Rijks Zwaan)	HR : ToMV/Fol;0,1/For/Pl/Va/Vd
6.	Unifort (De Ruiter)	HR: T0MV/Fol:0,1/For/Pl/Va/Vd/Ma/Mi/Mj ToMV/Ff:1-5/Fol:0,1/For/Va/Vd/Ma/Mi/Mj

HR - high resistance

IR - intermediate resistance

ToM - *Tomato Mosaic Virus*
V

Fol - *Fusarium oxysporum* f. sp *lycopersici*

For - *Fusarium oxysporum* f. sp. *radicis-lycopersici*

Pl - *Pyrenochaeta lycopersici*

Ff - *Fulvia fulva* (syn. *Cladosporium fulvum*)

Va - *Verticillium albo-atrum*

Vd - *Verticillium dahliae*

Ma - *Meloidogyne arenaria*

Mi - *Meloidogyne incognita*

Mj - *Meloidogyne javanlca*

Experiment design and statistical analysis

The experiment was a randomised block design with six-fold replication. Individual plots consisted of an island bed of 18 planting pots (36 plants) spaced at 50 cm (plot dimension was 9.5 m x 0.8 m). The six plots in a block were arranged along one row. The six blocks were arranged in adjacent bays of crop. Two heads were taken per plant to give a density of 4m^2. Results were examined by analysis of variance or regression analysis as appropriate.

Crop assessments

Plants were assessed at intervals to determine the number of wilting, yellowing and dead heads. When plant death was due to stem breakage or another above-ground factor, this was noted.

An initial assessment on crop appearance was done by the grower using the following categories:

Plant vigour – Low, medium, high (1-3)

Leaf scorch – A little, some, bad (1-3)

Severity of Mg deficiency on lower leaves – Low, medium, high (1-3)

Fruit size – Small, good (1-2)

Truss kink – None, a little, some (0-2)

Overall plant score – bottom (1-5) + top (1-5)

At subsequent assessments, plots were assessed for the incidence of heads affected by leaf necrosis or yellowing, the severity of leaf yellowing (nil, slight, moderate, severe) and the number of live heads per plot.

No assessment was possible at the end of cropping as the crop was pulled out early due to severe damage from russet mite.

Results and discussion

Crop assessments

At the initial assessment of crop appearance in early May, rootstock had no significant effect on leaf scorch, magnesium deficiency, fruit size or truss kink (Table 4.1). The vigour of Efialto was significantly greater than that of Beaufort at P = 0.063.

At the second assessment on 25 May, there were significant differences between rootstocks in leaf necrosis and leaf yellowing (Table 4.2). Leaf necrosis, which occurred mostly in the plant head, was relatively common (30-40% of plants) in cvs Stallone and Optifort, and significantly less in cvs Efialto, Beaufort and Unifort (9-14%). Leaf yellowing, which occurred mostly on lower and middle canopy leaves, occurred at a high incidence in cv. Unifort (47% of plants) and affected less than 29% of plants on all other rootstocks. However, the overall severity of leaf yellowing did not differ between rootstocks.

At the final assessment in mid-July, there were no significant differences between rootstocks in the incidence of plants with leaf yellowing, the severity of leaf yellowing or the surviving number of live heads per plot (Table 4.3).

Unfortunately no further assessments were possible due to a severe attack of russet mite which resulted in the crop being pulled out early.

Based on this work, there is some evidence that an Efialto rootstock results in greater plant vigour, less leaf necrosis and leaf yellowing than some other rootstocks early in the season in a crop of cv. Roterno grown in the soil.

Table 4.1: Effect of rootstock on appearance of soil-grown tomato crop, cv. Roterno – 7 May 2010

Rootstock	Plant vigour	Leaf scorch	Magnesium deficiency (1-3)	Fruit size (1-2)	Truss kink (0-2)	Overall plant score (0-10)
1. Beaufort	1.3	2.0	1.8	1.7	0.8	5.0
2. Efialto	2.5	2.3	1.8	1.7	1.5	6.7
3. Emperador	2.2	1.7	1.8	1.7	1.2	5.7
4. Optifort	2.2	1.8	2.5	1.7	1.0	5.3
5. Stallone	1.5	1.3	2.3	1.5	1.0	5.0
6. Unifort	1.7	1.5	1.5	1.8	0.7	5.2
Significance (25 df)	0.063	NS	NS	NS	NS	NS
SED	0.418	-	-	-	-	-

Table 4.2: Effect of tomato rootstock on appearance of soil-grown tomato, cv. Roterno, on six rootstocks – 25 May 2010

Rootstock	Mean % heads affected by		Leaf yellowing severity (0-3)
	Leaf necrosis (top leaves)	Leaf yellowing	
1. Beaufort	10.7 (4.7)	28.9 (8.2)	1.0
2. Efialto	8.9 (4.2)	15.2 (6.4)	0.6
3. Emperador	29.9 (6.7)	19.5 (7.2)	1.0
4. Optifort	39.8 (6.9)	9.3 (5.3)	0.8
5. Stallone	43.2 (6.9)	22.7 (7.6)	1.2
6. Unifort	14.1 (5.2)	46.6 (8.8)	1.4
Significance (25 df)	0.001	0.045	NS

NS - not significant; ( ) – standard error.

Table 4.3: Effect of tomato rootstock on appearance of soil-grown tomato, cv. Roterno, on six rootstocks – 14 July 2010

Rootstock	Mean number healthy heads per plot	Mean % plants with leaf yellowing	Leaf yellowing severity (0-3) and range
1. Beaufort	24.4	31	1.0 (0-2)
2. Efialto	24.8	16	1.2 (0-3)
3. Emperador	25.2	16	1.2 (1-2)
4. Optifort	25.2	9	0.6 (0-1)
5. Stallone	24.6	21	1.4 (0-3)
6. Unifort	25.4	19	1.4 (0-2)
Significance (25 df)	NS	NS	NS

NS - not significant; ( ) – standard error

T-RFLP analysis of roots

Analysis of rhizosphere microbial populations by T-RFLP found no significant effect of rootstock type or sample time on microbial diversity or species richness. The predominant fungi identified by FRAGSORT as possible pathogens on roots of the different rootstock, and according to sampling time, are shown in Table 4.4 and 4.5.

There appeared to be some possible rootstock effects worth further investigation, notably:

- Lower levels of *C. coccodes* on Beaufort, Emperador, Stallone and Unifort than Efialto and Optifort;
- Greater occurrence of *P. lycopersici/M. phaseolina* on Beaufort than the other varieties;
- Low level of *P. cucumerina* on Optifort than other varieties;
- Whether varieties differ in susceptibility to *Phytophthora* root rot.

Table 4.4: Occurrence and relative abundance (%) of selected fungal pathogens associated with roots of soil grown tomato according to rootstock

Likely fungal pathogen	TRF (Hae III)	T1 Bea	T2 Efi	T3 Emp	T4 Opt	T5 Sta	T6 Uni
Colletotrichum coccodes	153	3.6	13.6	4.8	14.6	7.2	5.7
Cylindrocarpon destructans	75	-	0.6	-	-	-	-
Fusarium solani	75	-	-	-	-	0.4	-
Fusarium sp.	73	-	0.7	-	-	-	-
Macrophomina phaseolina	327	12.0	5.6	7.6	2.2	7.2	3.6
Phytophthora cinnamomi	181	-	-	34.2	-	-	30.9
Plectosphaerella cucumerina	138	14.1	19.9	15.3	2.1	22.3	10.4
Pyrenochaeta lycopersici	328	12.0	5.6	7.6	2.2	7.2	3.6

Table 4.5: Occurrence and relative abundance (%) of selected fungal pathogens associated with roots of soil grown tomato according to sample time (crop age)

Likely fungal pathogens	TRF (Hae III)	TP1 (after planting)	TP2* (first pick)
C. coccodes	153	9.7	6.8
C. destructans	75	-	1.0
Fusarium solani	75	-	1.0
Fusarium sp.	73	-	0.8
M. phaseolina	327	8.8	3.9
P. cinnamomi	181	23.1	-
P. cucumerina	138	12.9	15.2
P. lycopersici	328	8.8	3.9

* T3 sampling was not possible due to early crop removal.

5. Effect of Trianum-P and Compete™ Plus on tomato root micro-organism populations

Introduction

There is increasing interest among growers in applying biological preparations containing micro-organisms to tomato roots with the aim of improving crop health. Information on the rhizosphere persistence of added micro-organisms, and their effect on the resident rhizosphere populations, is lacking. The aim of this experiment was to determine the effect of the single applications of Trianum-P and Compete™ Plus on the populations of micro-organisms associated with roots in a tomato crop grown in rockwool slabs.

Materials and Methods

This experiment was done in a commercial crop of cv. Star grown on rockwool slabs on a nursery in Norfolk in 2010. Trianum-P (*Trichoderma harzianum* T-22) and Compete™ Plus (containing *Bacillus, Pseudomonas, Streptomyces* and *Trichoderma* isolates) were each applied once at the label recommended rate to separate areas of crop in April 2010 via the drip irrigation system. Samples of nutrient solution (30 ml) and young roots (c. 5 g) were collected from slabs immediately before, and at up to 21 days after application (Table 5.1). Replicate root samples were taken from three adjacent slabs. Samples were tested for micro-organisms by T-RFLP tests at Nottingham University.

Table 5.1: Details of samples examined to determine effect of Trianum-P and Compete™ Plus on rhizosphere microbial populations – Norfolk, 2010

Sampling visit and date	Nature of samples taken from treatment areas		
	Untreated	Trianum-P	Compete Plus
Pre-treatment (20 Apr 2010)	-	Product	Product
At treatment (28 Apr 2010)	-	Solution at injection	Solution at injection
	-	Solution from dripper[1]	Solution from dripper[1]
	-	Young roots[2]	Young roots[2]
7 days after treatment (05 May 2010)	Young roots[3]	Young roots	Young roots
21 days after treatment (19 May 2010)	-	Young roots	Young roots

[1]Approximately 40-45 mins after injection, [2]Aproximately 1-1¼ hours after injection,

[3]Untreated control was from a different house and variety as all of the other blocks were treated with either Trianum-P or Compete™ Plus, sampled 06 May 2010.

Results and discussion

Compete™ Plus

Crops were sampled pre-application with CP (control), post CP treatment, 7 days after application and 21 days after application. There was no significant difference in fungal diversity between pre- and 7days after treatment; however the fungal diversity significantly reduced at 21 days. There was no significant difference in bacterial diversity between pre-treatment, post treatment, 7 days after treatment or 21 days after treatment samples (Figure 5.1).

Fungal species richness significantly reduced after the treatment, whereas bacterial species richness increased (Figure 5.2).

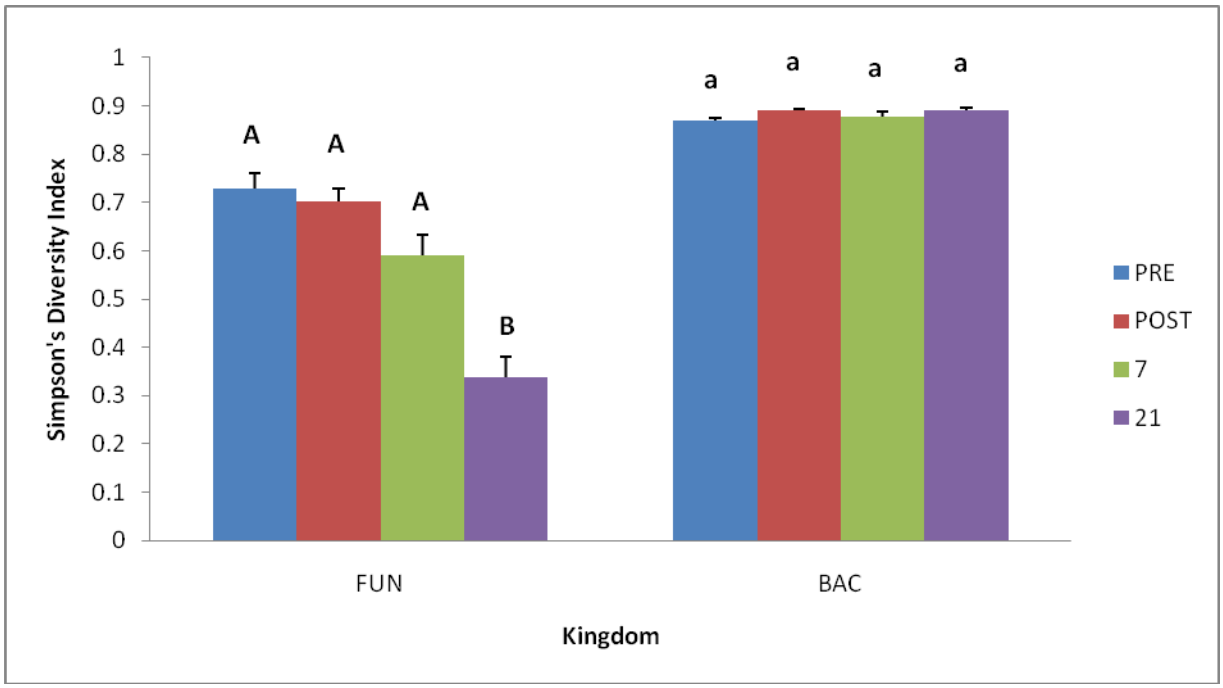

Figure 5.1. Effect of Compete™ Plus (CP) treatment on microbial diversity of the rhizosphere calculated using Simpson's Diversity Index. Error bars represent 1 standard error from the mean. Letters in common indicate samples are not significantly different.

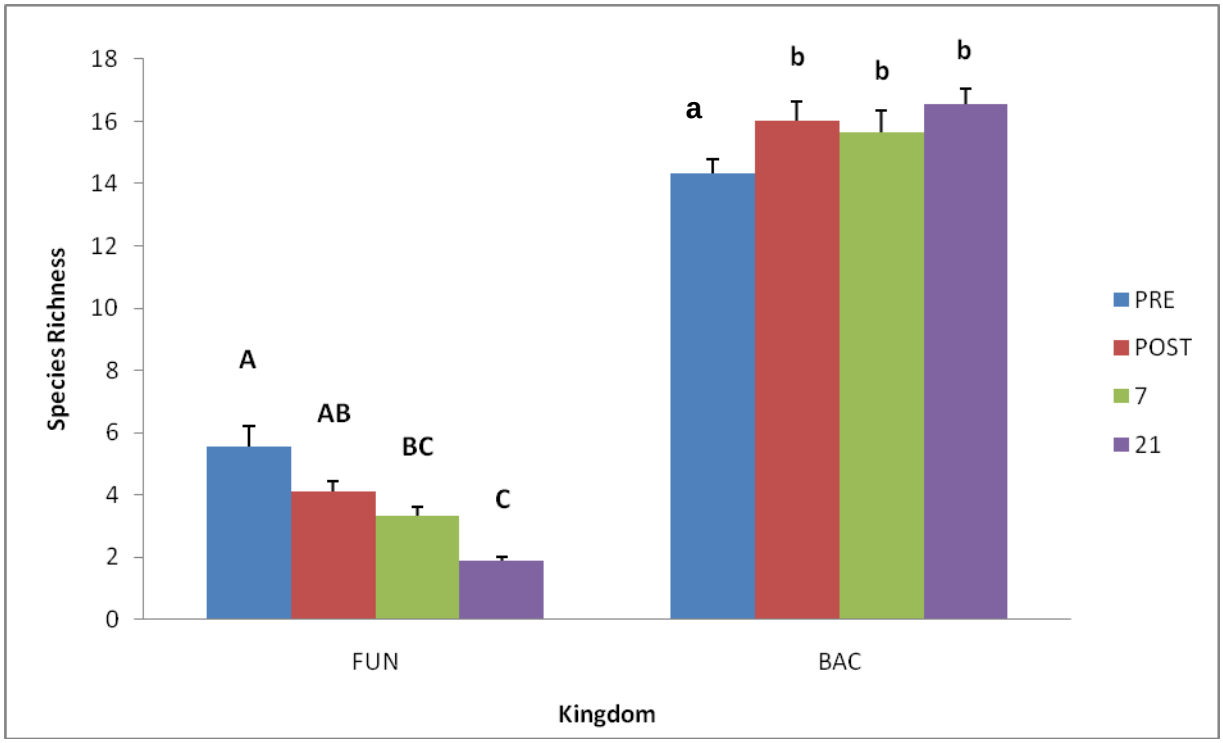

Figure 5.2. Effect of Compete™ Plus (CP) treatment on the species richness of the rhizosphere. Error bars represent 1 standard error from the mean. Letters in common indicate samples are not significantly different.

Fungal community

PCA showed that the first three principal components accounted for 94% of fungal diversity (Table 5.2).

PC1 and PC3 were significant between pre, post, 7 days and 21 days after treatment with CP samples (P= <0.05, P= <0.01 respectively), (Figure 5.3).

Two fungi identified as contributing to differences between sampling times were *G. rosae* and *P. cucumerina* (Table 5.3). These results indicate *G. rosae* contributes to the rhizosphere community at application but has reduced by 21 days after application.

Table 5.2: Results of principal component analysis of fungal T-RFLP data from pre, post, 7days and 21days after treatment with CP

Principal Component	PC1	PC2	PC3
Variation (%)	68.69	20.92	4.20
Cumulative (%)	68.69	89.61	93.81

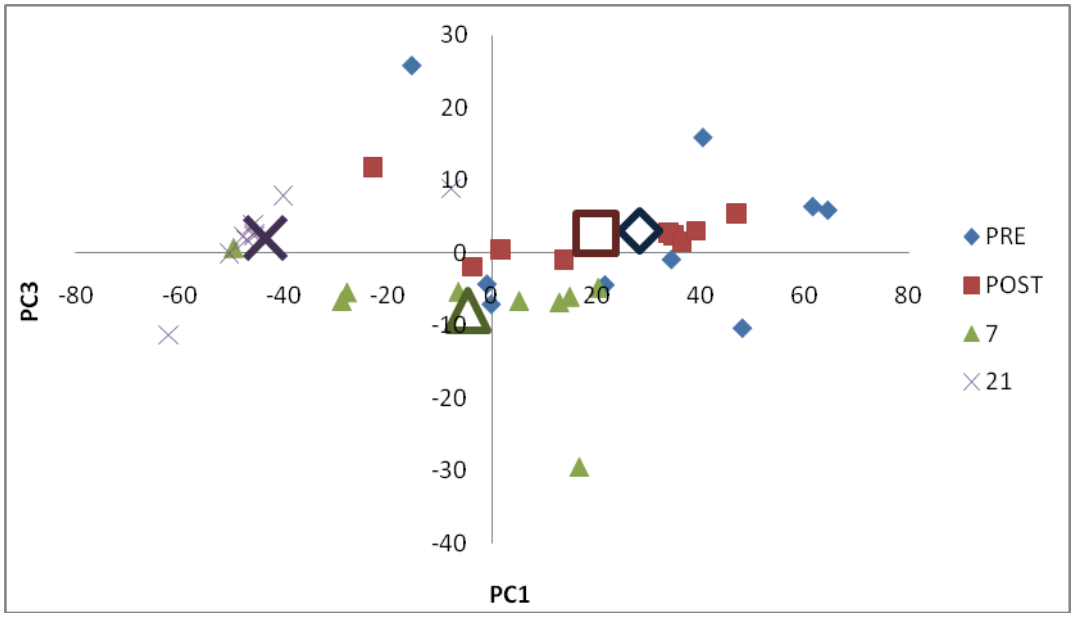

Figure 5.3. Ordination plot of PC1 versus PC3 scores using fungal relative abundance data of pre CP treatment (PRE), post CP treatment (POST), 7 days after treatment (7) and 21 days after treatment (21) samples.

Note: Large symbols represent mean PC score centroids.

Table 5.3: Significant fragment lengths contributing to PC1 and PC3, their possible identity and whether they are associated with pre CP treatment, post CP treatment, 7 days after and 21 days after treatment.

Fragment Length/Enzyme	PC1	PC3	Potential Identity	Associated with:
FLA_342	0.45	-	*Gigaspora rosae*	PRE/POST
FLA_385	-0.49	-0.51	Unknown	7/21
FLH_138	0.40	-	*Plectosphaerella cucumerina*	PRE/POST
FLH_182	-	0.32	Unknown	PRE/POST/21
FLH_207	-0.58	0.30	Unknown	ALL
FLH_342	-	-0.66	*Gigaspora rosae*	7/21

Bacterial community

PCA showed that the first three principal components accounts for 74% of bacterial community variation (Table 5.4). PC1, 2, 3 were significant for separating pre, post, 7 days and 21 days after treatment with CP (*P=0.1* for all).

Four bacteria were identified as contributing to differences between sampling times.

Table 5.4: Results of principal component analysis of bacterial T-RFLP data from pre, post, 7days and 21days after treatment with CP

Principal Component	PC1	PC2	PC3
Variation (%)	33.09	23.23	17.60
Cumulative (%)	33.09	56.32	73.92

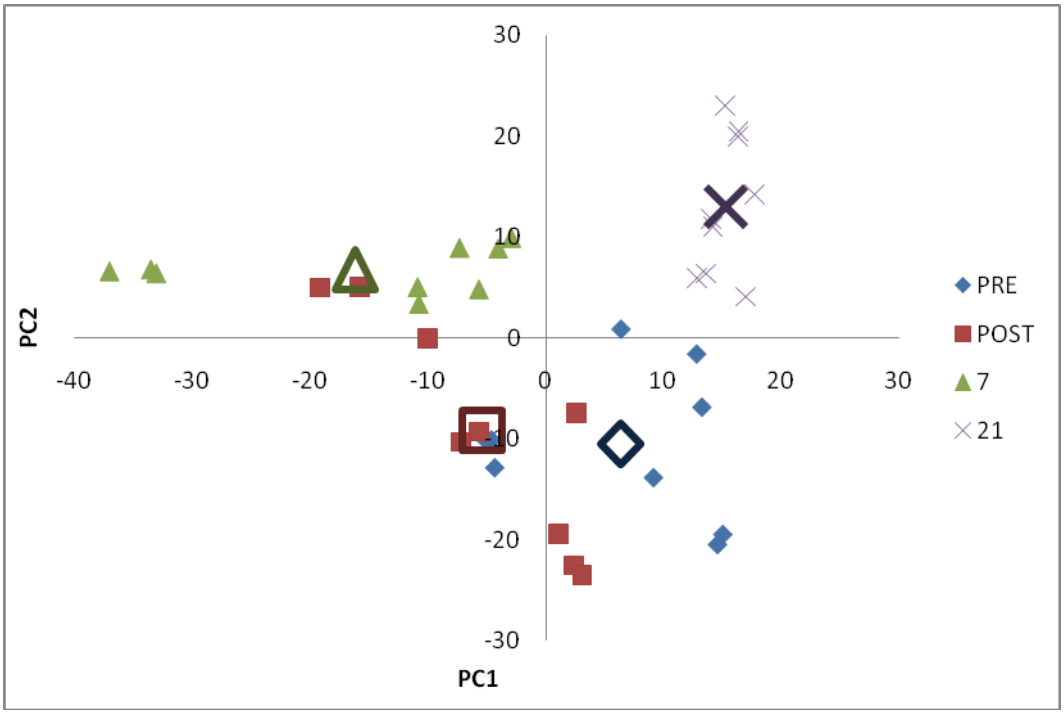

Figure 5.4. Ordination plot of PC1 versus PC2 scores using bacterial relative abundance data of pre CP treatment (PRE), post CP treatment (POST), 7days after treatment (7) and 21 days after treatment (21) samples.

Note: Large symbols represent mean PC score centroids.

T-RFLP examination of neat Compete Plus in water

One fungal organism and eight bacterial organisms were found to be present. It was not possible to identify the fungal species and multiple identities were indicated with the bacterial species.

6. Effect of a biological pre-plant treatment on soil micro-organisms

Introduction

A novel biological method of pre-plant soil disinfestation treatment known as anaerobic soil disinfestation (ASD) has recently been developed in the Netherlands. A defined organic substrate (e.g. rye grass) is incorporated into moist soil and covered with air impermeable film for four weeks. This treatment is reported to reduce soil levels of *V. dahliae*, other plant pathogens and nematodes, for high-value cropping (e.g. tree production). In 2010, a formulated protein rich mixture of by-products from potato, wheat and corn ('Herbie'), was marketed for use in organic tomato and pepper production as a treatment to 're-set' soils before planting (www.thatchtec/com). A UK tomato grower obtained dispensation from the Soil Association to test the treatment.

Opportunity was taken to examine the effects of Herbie treatment on soil micro-organism populations using the T-RFLP protocol developed in this project and other tests.

Materials and methods

Site and treatment details

The work was done on the Isle of Wight in a glasshouse that has grown organic tomatoes for over 5 years. Herbie 22 was applied at 1.5 kg/m^2 on 18 November, incorporated into the top 40 cm of soil and covered with Virtually Impermeable Film (VIF). The cover was removed on 15 December.

Soil samples

Soil samples were taken on 5 November 2010, just before crop removal, and from the same area on 15 December 2010, immediately after removal of the air-impermeable film. Soil cores were taken at 12 positions in each of three crop rows to 20 cm depth using a soil auger. The bulk soil samples were thoroughly mixed by tumbling it in a bucket and then divided into three portions for testing.

Soil tests

One sample was tested for *V. dahliae* at ADAS High Mowthorpe using the soil-sieving and selective agar method (Harris *et al.*, 2007). A second sample was tested for *V. dahliae* and *V. albo-atrum* using PCR tests developed at Fera in HDC project SF 97 (Peters and O'Neill, 2011). The third sample was tested for all fungi and bacteria using the T-RFLP method, used elsewhere in this project, at the University of Nottingham.

Results and discussion

The effect of 'Herbie' soil treatment on *Verticillium* spp. is shown in Table 6.1. No *V. dahliae* or *V. albo-atrum* were detected in any of the tests.

Table 6.1: Effect of 'Herbie' soil disinfestation on levels of *V. dahliae* and *V. albo-atrum* in soil – 2010

Soil treatment and sample	Agar test	Molecular test	
	V. dahliae (cfu/g)	*V. dahliae* (fg/g)	*V. albo-atrum* (fg/g)
Herbie			
Pre-treatment	Nil	<250	<250
Post-treatment	Not tested	<250	<250

Unfortunately no T-RFLP results were obtained on the samples due to failure to extract any DNA from the soils. This was likely due to a problem with an extraction buffer.

Overall conclusions

Literature reviews

1. A review of scientific literature indicates a world total of at least 66 fungal pathogens and 75 fungal saprotrophs have previously been found associated with tomato roots.
2. Root disease development is influenced by biotic and abiotic factors; effects are often complex due to interactions and results are sometimes contradictory.

Method development

3. Fungi are commonly present in and on tomato roots, including visibly healthy white roots, of crops grown in coir, soil, NFT, rockwool and woodfibre.
4. Fungi commonly present in or on the roots of UK commercial tomato crops (as determined by conventional plating) are *Colletotrichum coccodes*, *Fusarium* spp., *Pythium* spp., *Trichoderma* spp. and mucoraceous fungi. *Penicillium* spp. *and Thielaviopsis basicola* were isolated occasionally.
5. Root age influence occurrence of fungi on roots. *Fusarium* spp., pythiaceous fungi and *Penicillium* sp. were isolated more commonly from young thin roots than from older thicker roots, and *vice-versa* for *C. coccodes.*
6. T-RFLP is a molecular method providing the opportunity to identify relative quantities of individual fungal and bacterial species. Although T-RFLP can detect DNA in dead as well as live micro-organisms, it is generally considered that microbial DNA degrades rapidly in a biologically environment so that micro-organisms detected by T-RFLP are likely to be alive.
7. Direct extraction of DNA from tomato roots for a T-RFLP test gives more reliable and representative results than a wash extraction method, whilst keeping the benefits of having an internal semi-quantitative control due to the presence of plant material.
8. Bacterial primers 23Sfor and 23Srev* were found to be the best combination for obtaining a wide range of bacteria from tomato roots, and probably therefore a more representative picture of the bacterial community. This could be because these primers are better at binding to highly conserved regions of the 23S rRNA domain or that the other primers were homologous to regions that are less well conserved among bacterial organisms.

9. With regards to sampling techniques, the T-RFLP test showed that there was more diversity among microbial organisms on thin roots than medium and thick roots. These results could be explained by the thin roots being younger and actively growing, thus the site for most chemical activity and ultimately more attractive to more organisms.

10. T-RFLP test results from different root sampling methods for tomato grown in rockwool and NFT suggest that it makes little difference where you sample from within one slab or one row of a crop. This gives confidence that a relatively small number of samples can be taken for future studies whilst still obtaining representative results.

11. A molecular method such as T-RFLP is a distinct improvement over isolation onto agar for studying microbial communities and how components interact to determine whether or not disease develops; it is less time-consuming and provides more definitive identification. A database has been created that gives likely identification for 85 fungi and over 450 bacteria according to the DNA fragment length (TRF) produced using the restriction enzymes described in this work. TRFs for bacteria generally resulted in multiple potential identifications, meaning the method is less useful for studying bacterial communities. A microarray to study tomato rhizosphere communities would be a further improvement (see Conclusion 30).

Rhizosphere micro-organisms

12. Tomato crops in the UK may be affected by a wide range of root diseases. In 2009 and 2010 towards the end of cropping, visual examination of plants in 20 UK crops, combined with microscopic examination of root samples, confirmed brown and corky root rot (*Pyrenochaeta lycopersici*), black dot root rot (*Colletotrichum coccodes*), black root rot (*Thielaviopsis basicola*), Fusarium crown and root rot/Fusarium wilt (*Fusarium oxysporum*), Pythium root rot (*Pythium* spp.) and Verticillium wilt (*Verticillium albo-atrum*). T-RFLP tests indicated many more cases of likely infection by 12 potential fungal pathogens. Pathogens present in more than two crops were: *Plectosphaerella cucumerina* (17 crops), *Colletotrichum coccodes* (9), *Pythium* species (7), *Fusarium oxysporum* (6), *Pyrenochaeta lycopersici* (4) and *Phytophthora* species (4). The importance of *P. cucumerina* as a pathogen in UK crops warrants further examination.

13. Tomato rhizosphere microbial communities are often complex. Examination of root samples from the 20 crops by a molecular test (T-RFLP) indicated microbial

populations contained a total of over 100 fungal species and over 100 bacterial species.

14. Potential antagonists occur quite commonly on tomato roots including species of *Aspergillus, Penicillium, Gliocladium* and *Trichoderma*. *Gigaspora rosea,* an endomycorrhizal fungus that is known to be able to colonise tomato roots and may influence disease development, was found on plants in all five growing media.

15. Some fungi are present at relatively large abundance on tomato roots, as indicated by T-RFLP, notably *Colletotrichum coccodes*, *Macrophomina phaseolina*, *Fusarium oxysporum* and *Gigaspora rosea*. These fungi may have a role in root health and disease development more important than micro-organisms occurring at low levels.

Effect of growing medium and plant age on microbial diversity

16. Fungal and bacterial population diversity on tomato roots are significantly influenced by plant age and growing medium. Fungal population diversity on young roots from rockwool, coir and NFT crops increased with plant age, while that on soil crops decreased.

17. Rhizosphere microbial communities of tomato crops grown in rockwool, coir and woodfibre are quite similar to each other and distinct from both NFT and soil communities. Fungal diversity of root samples from rockwool, coir and woodfibre all clustered closely together and were distinct from those in soil and NFT.

Comparison of T-RFLP test and conventional tests

18. T-RFLP could be used as a diagnostic tool to test root samples for fungal pathogens and identify likely cause of root disease. However, there are method limitations (see below), and a microarray is likely to be a more suitable tool. T-RFLP tests on root samples indicated the presence at relatively high levels of several fungal pathogens which were confirmed by observation on diseased plants including *Colletotrichum coccodes, Fusarium oxysporum, Pythium* spp. and *Pyrenochaeta lycopersici.*

19. T-RFLP test results need to be interpreted using knowledge of UK tomato diseases and limitations of the test. Some root samples analysed by the FRAGSORT database indicated the presence of a few fungal pathogens which were unexpected, including *Phytophthora capsici* (not known to be present in the UK) and *Phytophthora cinnamomi*. The identity of these fragments needs to be re-examined by additional tests.

Effect of soil amendments on crop health, soil chemistry and microbial diversity

20. Soil biological amendments do not necessarily improve crop health. In a soil grown crop of cv. Piccolo on Beaufort rootstock, none of three pre-plant soil treatments (a composted green waste, Melcourt bark and Biofence) or two microbial treatments (Compete Plus drenches alternating with Colonize AG, and Trianum-P) significantly increased plant survival and root extent or reduced root discolouration and decay symptoms. Root disease levels were relatively low and potential benefits of these amendments on root health at higher levels of root disease cannot be discounted.

21. Green waste compost affects soil chemistry. Addition of a green waste compost to soil increased pH from 6.6 to 7.6, conductivity from 526 to 1140 µS/cm, and levels of chloride, sodium, potassium, nitrate, sulphate and boron. The compost also reduced leaf necrosis in the plant head, possibly due to moisture retention and greater availability to plants at times of temperature stress.

22. Bark affects soil chemistry. Addition of Melcourt Composted Fine Bark-FSC reduced levels of potassium and ammonium and increased levels of calcium and iron.

23. Soil microbial communities are not easily altered. None of the treatments affected microbial rhizosphere diversity, indicating this is difficult to alter within the duration of a soil grown crop by amendments or drench treatments.

24. Crop age significantly affects fungal diversity on roots. The abundance of *C. coccodes* increased greatly between first pick and July. The damage caused by black dot root rot to tomato warrants further investigation.

Effect of different rootstocks on crop health

25. Rootstock choice affects the relative abundance of different fungal pathogens on roots. We detected high levels of *C. coccodes* on Efialto and Optifort, and relatively high levels of *P. lycopersici* on Beaufort. It would be interesting to examine the effect of using different rootstocks over time on the build-up of fungal pathogens in soil. Rootstock choice had no significant effect on rhizosphere microbial diversity or species richness as measured by T-RFLP.

Effect of amendments on microbial diversity of rockwool tomato

26. Some microbial amendments can alter the microbial community in hydroponic tomato crops, at least temporarily. Addition of Compete™ Plus (various micro-

organisms) and Trianum P (*Trichoderma harzianum* strain T22) to nutrient solution of a rockwool crop was associated with increased bacterial species richness and decreased fungal species richness. We were unable to demonstrate that *Trichoderma* had established on roots.

Effect of UV treatment in NFT tomato

27. UV treatment of NFT solution is unlikely to control black dot root rot. A UV treatment of recycled solution in an NFT crop did not control *Colletotrichum coccodes* or *Plectosphaerella cucumerina* as determined by T-RFLP tests. This method of water treatment is more likely to be effective against oomycetes pathogens (eg *Pythium* sp.).
28. *Pythium dissocotum* appears to be an important pathogen of hydroponic tomato crops. We isolated this species from roots of an NFT tomato crop. Previous studies in France have shown a predominance of this species in tomatoes grown in soilless culture.

Disease prediction

29. We are unable to predict tomato root health or disease using T-RFLP. There was no obvious association between fungal and bacterial diversity (measured by Simpson's Diversity Index) on roots during cropping, and 'plant sickness' or 'root rot' at the end of cropping. More extensive work is required before determining conclusively whether T-RFLP can be used to predict root health or disease. It is recommended that further effort in this area should focus on one variety and one growing medium in order to reduce sources of variation and enhance prospects for detecting any relationship.

New test for root micro-organisms

30. A microarray test is likely to be a better tool than T-RFLP to investigate possible relationships between micro-organism occurrence on roots and root health or disease. A suitable microarray can identify micro-organisms to species level with a higher degree of certainty than T-RFLP and provides semi-quantitative information on abundance. A microarray for detection and quantification of around 50 tomato rhizosphere fungi (pathogens and potential beneficial micro-organisms) is being developed. This project has been used to inform which species are included on the microarray. Microarray test validation will be done in 2012.

Technology transfer

Publications

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Presentations

Tomato root health-project objectives, Wight Salads Group, Isle of Wight, 23 July 2008 (Tim O'Neill).

Project progress report, Wight Salad Group, Isle of Wight, 30 October 2008 (Tim O'Neill).

Root diseases and their control. WSG Kent, 28 May 2009 (Tim O'Neill).

Tomato disease research update. WSG Isle of Wight, 14 July 2009 (Tim O'Neill)

Monitoring tomato root micro-organisms – a step towards disease prediction and control. Annual TGA Conference, Coventry, 30 September 2010 (Tim O'Neill and Sarah Deery)

Project meetings

Project start-up meeting, ADAS Arthur Rickwood, 10 April 2008.

T-RFLP techniques meeting with Warwick HRI, Sutton Bonington, 1 October 2008.

Project progress meeting, Sutton Bonington, 8 October 2008.

Statistics meeting, Sutton Bonington, 24 October 2008.

Project review meeting, Cornerways Nursery, Norfolk, 8 April 2009.

Project review meeting, Sutton Bonington, 9 October 2009.

Project review meeting, Arreton Valley Nursery, Isle of Wight, 13 April 2010

Project review meeting, Cornerways Nursery, Norfolk, 20 October 2010

Project review meeting, Sutton Bonington, 19 May 2011

Glossary

Deoxyribonucleic acid (DNA): a nucleic acid that carries the genetic information in cells and is used in the development and functioning of all known living organisms

FRAGSORT: a computer sorting tool for the analysis of T-RFLP data

Microarray: consists of a large number of DNA probes attached to a solid surface, to which labelled targeted amplified DNA hybridizes. If targeted DNA is present a fluorescent signal is detected on the corresponding probes allowing for high throughput detection of targets and their relative abundance

Polymerase Chain Reaction (PCR): a molecular technique which allows the production of large quantities of a specific DNA region from a DNA template using an enzymatic reaction

Principal component analysis (PCA): a mathematical procedure that allows you to identify patterns in data, and express the data in such a way that highlights similarities and differences within the dataset

Relative species abundance: refers to how common or rare a species is relative to other species in a community

Restriction enzymes (RE): an enzyme that can recognize and cleave specific sequences in DNA to produce discrete fragments

Simpson's Diversity Index: a composite equation which takes into account the number of species present, as well as the relative abundance of each species

Species diversity: accounts for the number of species in an area and also their relative abundance

Species richness: the number of individual species in a community

Terminal Restriction Fragment Length Polymorphism (T-RFLP): a molecular technique for profiling microbial communities based on the position of restriction sites closest to a labelled end of an amplified target gene

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Appendix 1 – FRAGSORT fungal identifications – crop monitoring 2010

Rockwool 1

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Cladosporium herbarum	322	7.27204	322	1.12812	1.12812
Colletotrichum coccodes	189	2.88946	153	17.18109	2.88946
Gigaspora rosae	342	33.67605	342	8.17177	8.17177
Gigaspora spp	348	0.39369	348	3.67774	0.39369
Plectosphaerella	343	33.67605	138	21.84922	21.84922
Plectosphaerella cucumerina	342	33.67605	138	21.84922	21.84922
Rhynchosporium secalis	182	0.62435	147	1.30307	0.62435

Rockwool 2

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Cladosporium herbarum	322	0.84346	322	0.30867	0.30867
Cylindrocarpon destructans	119	4.0788	75	19.8313	4.0788
Fusarium culmorum	120	4.0788	73	19.8313	4.0788
Fusarium oxysporum	120	4.0788	73	19.8313	4.0788
Fusarium oxysporum f. sp. Lycopersici	120	4.0788	73	19.8313	4.0788
Fusarium oxysporum f. sp. radicis-lycopersici	120	4.0788	73	19.8313	4.0788
Fusarium redolens	120	4.0788	73	19.8313	4.0788
Gigaspora rosae	342	27.48006	342	10.6084	10.60839
Plectosphaerella	343	27.48006	138	19.2214	19.22139
Plectosphaerella cucumerina	342	27.48006	138	19.2214	19.22139

Soil 1

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Candida albicans	324	36.76121	324	19.39524	19.39524
Chaetomium bostrychodes	326	1.90198	70	2.28401	1.90198
Colletotrichum acutatum	329	0.40769	154	18.37653	0.40769
Colletotrichum coccodes	189	1.65867	153	18.37653	1.65867
Epicoccum nigrum - isolate M7	189	1.65867	328	0.35372	0.35372
Epicoccum spp	190	1.65867	329	0.35372	0.35372
Gigaspora rosae	342	6.56137	342	2.74853	2.74853
Leptosphaeria spp.	188	1.65867	333	0.32603	0.32603
Macrophomina phaseolina	188	1.65867	327	0.35372	0.35372
Penicillium purpurogenum	329	0.40769	329	0.35372	0.35372
Plectosphaerella	343	6.56137	138	4.56996	4.56996
Plectosphaerella cucumerina	342	6.56137	138	4.56996	4.56996
Pyrenochaeta (Phoma) lycopersici	189	1.65867	328	0.35372	0.35372
Verticillium albo-atrum	343	6.56137	134	1.39802	1.39802
Verticillium tricorpus	339	6.56137	134	1.39802	1.39802

Soil 2

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Aspergillus flavus	340	23.26897	81	0.43755	0.43755
Aspergillus fumigatus	339	23.26897	81	0.43755	0.43755
Aspergillus sydowii	339	23.26897	83	7.33273	7.33273
Aspergillus ustus	341	23.26897	85	7.33273	7.33273
Candida albicans	324	2.21	324	1.46974	1.46974
Cephalosporium spp.	336	2.30433	80	0.43755	0.43755
Cladosporium cladosporioides	323	27.22511	323	1.22565	1.22565
Cladosporium herbarum	322	27.22511	322	1.22565	1.22565
Colletotrichum acutatum	329	16.57565	154	24.88444	16.57565
Gigaspora rosae	342	23.26897	342	7.00847	7.00847
Gigaspora spp	348	5.68106	348	0.31449	0.31449
Gliocladium spp.	341	23.26897	156	2.3587	2.3587
Penicillium chrysogenum	333	2.30433	80	0.43755	0.43755
Penicillium italicum	336	2.30433	80	0.43755	0.43755
Penicillium jensenii	337	2.30433	80	0.43755	0.43755
Penicillium lividum	335	2.30433	79	0.43755	0.43755
Penicillium purpurogenum	329	16.57565	329	2.46156	2.46156
Penicillium thomii	332	16.57565	81	0.43755	0.43755
Penicillium variabile	346	5.68106	71	8.09735	5.68106
Plectosphaerella	343	23.26897	138	11.93429	11.93429
Plectosphaerella cucumerina	342	23.26897	138	11.93429	11.93429
Trichoderma viride	349	5.68106	155	24.88444	5.68106
Verticillium albo-atrum	343	23.26897	134	0.52814	0.52814
Verticillium tricorpus	339	23.26897	134	0.52814	0.52814
Volutella ciliata	86	1.44285	342	7.00847	1.44285

NFT 1

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Blastocladiella emersonii	206	3.5442	258	3.88381	3.5442
Gigaspora rosae	342	6.09389	342	8.33939	6.09389
Plectosphaerella	343	6.09389	138	22.35865	6.09389
Plectosphaerella cucumerina	342	6.09389	138	22.35865	6.09389

NFT 2

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Aspergillus flavus	340	25.35819	81	0.95659	0.95659
Aspergillus fumigatus	339	25.35819	81	0.95659	0.95659
Aspergillus niger	340	25.35819	82	0.95659	0.95659
Aspergillus sydowii	339	25.35819	83	0.95659	0.95659
Calyptella capula	81	1.8079	370	1.98277	1.8079
Chaetomium bostrychodes	326	0.54947	70	2.70093	0.54947
Colletotrichum acutatum	329	0.74487	154	33.8114	0.74487
Colletotrichum coccodes	189	2.59145	153	33.8114	2.59145
Cylindrocarpon destructans	119	0.15575	75	3.59145	0.15575
Fusarium culmorum	120	0.15575	73	1.05121	0.15575
Fusarium oxysporum	120	0.15575	73	1.05121	0.15575
Fusarium oxysporum f. sp. Lycopersici	120	0.15575	73	1.05121	0.15575
Fusarium oxysporum f. sp. radicis-lycopersici	120	0.15575	73	1.05121	0.15575
Fusarium redolens	120	0.15575	73	1.05121	0.15575
Fusarium solani	105	4.08702	75	3.59145	3.59145
Gigaspora rosae	342	25.35819	342	5.79004	5.79004
Gliocladium roseum	339	25.35819	119	0.76686	0.76686
Glomus intraradices	378	2.03418	378	2.34797	2.03418
Humicola fuscoatra	188	2.59145	81	0.95659	0.95659
Mortierella alpina	119	0.15575	58	0.20905	0.15575
Mortierella spp	119	0.15575	58	0.20905	0.15575
Mucor meihei	93	3.72516	69	2.70093	2.70093
Penicillium griseofulvum	340	25.35819	82	0.95659	0.95659
Penicillium janthinellum	341	25.35819	82	0.95659	0.95659
Penicillium thomii	332	0.74487	81	0.95659	0.74487
Plectosphaerella	343	25.35819	138	19.49772	19.49772
Plectosphaerella cucumerina	342	25.35819	138	19.49772	19.49772
Rhynchosporium secalis	182	2.04145	147	0.31479	0.31479

Coir 1

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Aspergillus flavus	340	1.7357	81	4.98403	1.7357
Aspergillus fumigatus	339	5.68126	81	4.98403	4.98403
Aspergillus niger	340	1.7357	82	4.98403	1.7357
Aspergillus sydowii	339	5.68126	83	4.98403	4.98403
Candida albicans	324	26.57489	324	1.29152	1.29152
Chaetomium bostrychodes	115	1.10991	78	1.76802	1.10991
Cladosporium cladosporioides	323	26.57489	323	1.29152	1.29152
Cladosporium herbarum	322	26.57489	322	0.23635	0.23635
Colletotrichum acutatum	329	1.79814	154	40.95899	1.79814
Gigaspora rosae	342	1.7357	342	8.58901	1.7357
Gigaspora spp	348	0.32256	348	1.00715	0.32256
Paecilomyces lilacinus	334	3.13931	183	1.48148	1.48148
Penicillium digitatum	338	0.43739	82	4.98403	0.43739
Penicillium griseofulvum	340	1.7357	82	4.98403	1.7357
Penicillium janthinellum	341	1.7357	82	4.98403	1.7357
Penicillium purpurogenum	329	1.79814	329	0.58761	0.58761
Penicillium thomii	332	3.13931	81	4.98403	3.13931
Penicillium verrucosum	338	0.43739	81	4.98403	0.43739
Plectosphaerella	343	1.7357	138	3.06101	1.7357
Plectosphaerella cucumerina	342	1.7357	138	3.06101	1.7357
Rhizopus oryzae	361	0.38094	361	0.7087	0.38094
Spongospora subterranea	153	1.01678	325	1.29152	1.01678
Trichoderma viride	349	0.32256	155	40.95899	0.32256

Coir 2

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Aspergillus flavus	340	11.95404	81	1.43741	1.43741
Aspergillus fumigatus	339	0.4448	81	1.43741	0.4448
Aspergillus niger	340	11.95404	82	1.43741	1.43741
Botrytis cinerea	175	0.14911	319	1.34573	0.14911
Botrytis fabae	175	0.14911	319	1.34573	0.14911
Calyptella capula	81	0.22689	370	0.41695	0.22689
Cephalosporium spp.	336	0.84834	80	1.43741	0.84834
Cladosporium herbarum	322	12.82403	322	1.34573	1.34573
Colletotrichum acutatum	329	0.37282	154	20.43825	0.37282
Gigaspora rosae	342	4.31461	342	8.86068	4.31461
Paecilomyces lilacinus	334	0.22345	183	3.6289	0.22345
Penicillium chrysogenum	333	0.22345	80	1.43741	0.22345
Penicillium digitatum	338	0.4448	82	1.43741	0.4448
Penicillium griseofulvum	340	11.95404	82	1.43741	1.43741
Penicillium italicum	336	0.84834	80	1.43741	0.84834
Penicillium janthinellum	341	11.95404	82	1.43741	1.43741
Penicillium jensenii	337	0.84834	80	1.43741	0.84834
Penicillium thomii	332	0.59465	81	1.43741	0.59465
Penicillium verrucosum	338	0.4448	81	1.43741	0.4448
Phytophthora cinnamomi	177	0.14911	181	3.6289	0.14911
Plectosphaerella	343	4.31461	138	12.30563	4.31461
Plectosphaerella cucumerina	342	4.31461	138	12.30563	4.31461
Rhizopus oryzae	361	5.46704	361	7.14978	5.46704

Woodfibre 1

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Aspergillus flavus	340	10.03229	81	2.03181	2.03181
Aspergillus fumigatus	339	10.03229	81	2.03181	2.03181
Aspergillus niger	340	10.03229	82	2.03181	2.03181
Aspergillus sydowii	339	10.03229	83	2.03181	2.03181
Aspergillus ustus	341	10.03229	85	1.52084	1.52084
Colletotrichum acutatum	329	2.7862	154	7.36262	2.7862
Colletotrichum coccodes	189	2.92145	153	7.36262	2.92145
Gigaspora rosae	342	10.03229	342	0.37023	0.37023
Humicola fuscoatra	188	2.92145	81	2.03181	2.03181
Leptosphaeria spp.	188	2.92145	333	0.21826	0.21826
Paecilomyces lilacinus	334	0.24159	183	2.4627	0.24159
Penicillium digitatum	338	2.57172	82	2.03181	2.03181
Penicillium griseofulvum	340	10.03229	82	2.03181	2.03181
Penicillium janthinellum	341	10.03229	82	2.03181	2.03181
Penicillium thomii	332	0.24159	81	2.03181	0.24159
Penicillium verrucosum	338	2.57172	81	2.03181	2.03181
Plectosphaerella	343	10.03229	138	5.46735	5.46735
Plectosphaerella cucumerina	342	10.03229	138	5.46735	5.46735
Rhizopus oryzae	361	4.02967	361	7.2428	4.02967

Woodfibre 2

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Cylindrocarpon destructans	119	1.40441	75	15.86634	1.40441
Fusarium culmorum	120	1.40441	73	8.48547	1.40441
Fusarium oxysporum	120	1.40441	73	8.48547	1.40441
Fusarium oxysporum f. sp. Lycopersici	120	1.40441	73	8.48547	1.40441
Fusarium oxysporum f. sp. radicis-lycopersici	120	1.40441	73	8.48547	1.40441
Fusarium redolens	120	1.40441	73	8.48547	1.40441
Gigaspora rosae	342	0.49086	342	0.42321	0.42321
Gigaspora spp	348	2.16739	348	0.48372	0.48372
Gliocladium spp.	341	0.49086	156	1.78225	0.49086
Paecilomyces lilacinus	334	9.64769	183	3.30804	3.30804
Penicillium variabile	346	0.18371	71	8.48547	0.18371
Plectosphaerella	343	0.49086	138	0.68028	0.49086
Plectosphaerella cucumerina	342	0.49086	138	0.68028	0.49086
Rhizopus oryzae	361	16.60376	361	3.4519	3.4519
Thielaviopsis basicola	101	15.99763	157	10.3204	10.3204
Trichoderma viride	349	4.66564	155	1.78225	1.78225

Appendix 2 – FRAGSORT output for soil amendment trial

Fungal treatment

T1

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Candida albicans	324	9.35693	324	1.23294	1.23294
Cladosporium cladosporioides	323	28.3111	323	1.23294	1.23294
Cladosporium herbarum	322	28.3111	322	1.23294	1.23294
Colletotrichum coccodes	189	1.58849	153	36.64142	1.58849
Epicoccum nigrum - isolate M7	189	1.58849	328	9.83445	1.58849
Epicoccum spp	190	1.58849	329	0.13544	0.13544
Gigaspora rosae	342	13.66348	342	1.8913	1.8913
Gliocladium roseum	339	13.66348	119	0.25431	0.25431
Glomus intraradices	378	2.21091	378	1.71895	1.71895
Glomus mossae	396	0.23462	396	0.22812	0.22812
Leptosphaeria spp.	188	1.58849	333	0.13544	0.13544
Macrophomina phaseolina	188	1.58849	327	9.83445	1.58849
Paecilomyces lilacinus	334	0.65784	183	1.11691	0.65784
Plectosphaerella	343	13.66348	138	10.79433	10.79433
Plectosphaerella cucumerina	342	13.66348	138	10.79433	10.79433
Pyrenochaeta (Phoma) lycopersici	189	1.58849	328	9.83445	1.58849

T2

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Aspergillus sydowii	339	10.93738	83	0.38333	0.38333
Aspergillus ustus	341	10.93738	85	0.38333	0.38333
Candida albicans	324	9.41784	324	1.17437	1.17437
Cladosporium cladosporioides	323	24.73694	323	1.17437	1.17437
Cladosporium herbarum	322	24.73694	322	1.17437	1.17437
Colletotrichum coccodes	189	1.00976	153	40.94899	1.00976
Epicoccum nigrum - isolate M7	189	1.00976	328	8.01014	1.00976
Epicoccum spp	190	1.00976	329	1.60592	1.00976
Gigaspora rosae	342	10.93738	342	1.49001	1.49001
Leptosphaeria spp.	188	1.00976	333	1.60592	1.00976
Macrophomina phaseolina	188	1.00976	327	8.01014	1.00976
Plectosphaerella	343	10.93738	138	6.76868	6.76868
Plectosphaerella cucumerina	342	10.93738	138	6.76868	6.76868
Pyrenochaeta (Phoma) lycopersici	189	1.00976	328	8.01014	1.00976

T3

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Candida albicans	324	6.04452	324	1.25391	1.25391
Cladosporium cladosporioides	323	25.22795	323	1.25391	1.25391
Cladosporium herbarum	322	25.22795	322	1.25391	1.25391
Colletotrichum coccodes	189	2.72552	153	43.58208	2.72552
Epicoccum nigrum - isolate M7	189	2.72552	328	7.87928	2.72552
Epicoccum spp	190	2.72552	329	1.81923	1.81923
Gigaspora rosae	342	12.57588	342	1.60744	1.60744
Gigaspora spp	348	1.5682	348	2.33751	1.5682
Gliocladium roseum	339	12.57588	119	1.73769	1.73769
Glomus intraradices	378	1.40213	378	0.31077	0.31077
Leptosphaeria spp.	188	2.72552	333	1.81923	1.81923
Macrophomina phaseolina	188	2.72552	327	7.87928	2.72552
Paecilomyces lilacinus	334	2.39209	183	0.95427	0.95427
Penicillium variabile	346	1.5682	71	1.49334	1.49334
Plectosphaerella	343	12.57588	138	8.69619	8.69619
Plectosphaerella cucumerina	342	12.57588	138	8.69619	8.69619
Pyrenochaeta (Phoma) lycopersici	189	2.72552	328	7.87928	2.72552

T4

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Aspergillus sydowii	339	6.55054	83	5.94195	5.94195
Aspergillus ustus	341	6.55054	85	5.94195	5.94195
Botrytis cinerea	175	0.19433	319	1.33889	0.19433
Botrytis fabae	175	0.19433	319	1.33889	0.19433
Candida albicans	324	0.88538	324	1.1677	0.88538
Cladosporium cladosporioides	323	25.17909	323	1.1677	1.1677
Cladosporium herbarum	322	25.17909	322	1.1677	1.1677
Colletotrichum coccodes	189	1.9446	153	33.53143	1.9446
Epicoccum nigrum - isolate M7	189	1.9446	328	0.7659	0.7659
Epicoccum spp	190	1.9446	329	0.70848	0.70848
Gigaspora rosae	342	6.55054	342	0.70292	0.70292
Gigaspora spp	348	3.08643	348	1.83688	1.83688
Gliocladium roseum	339	6.55054	119	0.47588	0.47588
Leptosphaeria spp.	188	1.9446	333	0.70848	0.70848
Macrophomina phaseolina	188	1.9446	327	0.7659	0.7659
Paecilomyces lilacinus	334	2.16404	183	0.8291	0.8291
Penicillium variabile	346	3.08643	71	6.57267	3.08643
Plectosphaerella	343	6.55054	138	3.29133	3.29133
Plectosphaerella cucumerina	342	6.55054	138	3.29133	3.29133
Pyrenochaeta (Phoma) lycopersici	189	1.9446	328	0.7659	0.7659
Sclerotinia cepivorum	174	0.19433	318	1.33889	0.19433
Sclerotinia sclerotiorum	174	0.19433	318	1.33889	0.19433
Verticillium nigrescens	344	3.08643	84	5.94195	3.08643

T5

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Aspergillus sydowii	339	2.13567	83	1.30095	1.30095
Aspergillus ustus	341	2.13567	85	1.30095	1.30095
Botrytis cinerea	175	1.12434	319	2.2728	1.12434
Botrytis fabae	175	1.12434	319	2.2728	1.12434
Candida albicans	324	2.89842	324	1.19607	1.19607
Cladosporium cladosporioides	323	25.89676	323	1.19607	1.19607
Cladosporium herbarum	322	25.89676	322	1.19607	1.19607
Colletotrichum coccodes	189	1.70044	153	39.11997	1.70044
Epicoccum nigrum - isolate M7	189	1.70044	328	2.01826	1.70044
Epicoccum spp	190	1.70044	329	0.88426	0.88426
Gigaspora rosae	342	2.13567	342	0.22767	0.22767
Gigaspora spp	348	0.92403	348	0.5473	0.5473
Gliocladium roseum	339	2.13567	119	0.76592	0.76592
Gliocladium spp.	341	2.13567	156	0.94887	0.94887
Glomus intraradices	378	1.05036	378	0.32108	0.32108
Leptosphaeria spp.	188	1.70044	333	0.88426	0.88426
Macrophomina phaseolina	188	1.70044	327	2.01826	1.70044
Paecilomyces lilacinus	334	1.77578	183	1.9611	1.77578
Penicillium variabile	346	0.92403	71	2.10988	0.92403
Plectosphaerella	343	2.13567	138	1.13154	1.13154
Plectosphaerella cucumerina	342	2.13567	138	1.13154	1.13154
Pyrenochaeta (Phoma) lycopersici	189	1.70044	328	2.01826	1.70044
Sclerotinia cepivorum	174	1.12434	318	2.2728	1.12434
Sclerotinia sclerotiorum	174	1.12434	318	2.2728	1.12434
Verticillium nigrescens	344	0.92403	84	1.30095	0.92403

T6

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Aspergillus sydowii	339	5.3416	83	1.53685	1.53685
Aspergillus ustus	341	5.3416	85	1.53685	1.53685
Botrytis cinerea	175	0.41346	319	0.67028	0.41346
Botrytis fabae	175	0.41346	319	0.67028	0.41346
Candida albicans	324	2.65333	324	2.26742	2.26742
Cladosporium cladosporioides	323	29.30477	323	2.26742	2.26742
Cladosporium herbarum	322	29.30477	322	2.26742	2.26742
Colletotrichum coccodes	189	2.61659	153	45.46218	2.61659
Epicoccum nigrum - isolate M7	189	2.61659	328	2.57832	2.57832
Epicoccum spp	190	2.61659	329	2.57832	2.57832
Gigaspora rosae	342	5.3416	342	0.50152	0.50152
Gigaspora spp	348	0.32439	348	0.56307	0.32439
Glomus mossae	396	0.70574	396	0.56107	0.56107
Macrophomina phaseolina	188	2.61659	327	2.57832	2.57832
Paecilomyces lilacinus	334	0.49931	183	0.25716	0.25716
Penicillium variabile	346	0.32439	71	2.00348	0.32439
Plectosphaerella	343	5.3416	138	0.9267	0.9267
Plectosphaerella cucumerina	342	5.3416	138	0.9267	0.9267
Pyrenochaeta (Phoma) lycopersici	189	2.61659	328	2.57832	2.57832
Sclerotinia cepivorum	174	0.41346	318	0.67028	0.41346
Sclerotinia sclerotiorum	174	0.41346	318	0.67028	0.41346
Verticillium nigrescens	344	0.32439	84	1.53685	0.32439

Time point (TP)

FTP1

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Aspergillus sydowii	339	14.4281	83	0.64127	0.64127
Aspergillus ustus	341	14.4281	85	0.64127	0.64127
Botrytis cinerea	175	0.65933	319	1.90738	0.65933
Botrytis fabae	175	0.65933	319	1.90738	0.65933
Cladosporium herbarum	322	4.74229	322	1.90738	1.90738
Colletotrichum coccodes	189	0.227	153	7.84365	0.227
Epicoccum nigrum - isolate M7	189	0.227	328	5.78458	0.227
Epicoccum spp	190	0.227	329	0.39419	0.227
Gigaspora rosae	342	14.4281	342	1.58433	1.58433
Gliocladium spp.	341	14.4281	156	0.47444	0.47444
Glomus intraradices	378	3.45163	378	1.1754	1.1754
Leptosphaeria spp.	188	0.227	333	0.39419	0.227
Macrophomina phaseolina	188	0.227	327	5.78458	0.227
Paecilomyces lilacinus	334	0.31422	183	0.21192	0.21192
Plectosphaerella	343	14.4281	138	8.072	8.072
Plectosphaerella cucumerina	342	14.4281	138	8.072	8.072
Pyrenochaeta (Phoma) lycopersici	189	0.227	328	5.78458	0.227
Sclerotinia cepivorum	174	0.65933	318	1.90738	0.65933
Sclerotinia sclerotiorum	174	0.65933	318	1.90738	0.65933

FTP2

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Aspergillus sydowii	339	2.87138	83	1.94071	1.94071
Aspergillus ustus	341	2.87138	85	1.94071	1.94071
Botrytis cinerea	175	0.20673	319	1.05754	0.20673
Botrytis fabae	175	0.20673	319	1.05754	0.20673
Candida albicans	324	1.74019	324	1.49419	1.49419
Cladosporium cladosporioides	323	15.5457	323	1.49419	1.49419
Cladosporium herbarum	322	15.5457	322	1.49419	1.49419
Colletotrichum coccodes	189	0.15543	153	44.74329	0.15543
Epicoccum nigrum - isolate M7	189	0.15543	328	3.38137	0.15543
Epicoccum spp	190	0.15543	329	2.18247	0.15543
Gigaspora rosae	342	2.87138	342	0.94787	0.94787
Leptosphaeria spp.	188	0.15543	333	2.18247	0.15543
Macrophomina phaseolina	188	0.15543	327	3.38137	0.15543
Paecilomyces lilacinus	334	0.43949	183	3.10278	0.43949
Plectosphaerella	343	2.87138	138	2.79935	2.79935
Plectosphaerella cucumerina	342	2.87138	138	2.79935	2.79935
Pyrenochaeta (Phoma) lycopersici	189	0.15543	328	3.38137	0.15543
Sclerotinia cepivorum	174	0.20673	318	1.05754	0.20673
Sclerotinia sclerotiorum	174	0.20673	318	1.05754	0.20673

FTP3

Organism Identity	Alu I TRF	Alu I (%)	Hae III TRF	Hae III (%)	Min (%)
Aspergillus sydowii	339	8.30279	83	1.99956	1.99956
Aspergillus ustus	341	8.30279	85	1.99956	1.99956
Candida albicans	324	6.66045	324	2.65202	2.65202
Cladosporium cladosporioides	323	59.04031	323	2.65202	2.65202
Cladosporium herbarum	322	59.04031	322	2.65202	2.65202
Colletotrichum coccodes	189	5.41028	153	67.0561	5.41028
Epicoccum nigrum - isolate M7	189	5.41028	328	6.37722	5.41028
Epicoccum spp	190	5.41028	329	6.37722	5.41028
Gigaspora rosae	342	8.30279	342	0.67822	0.67822
Gigaspora spp	348	2.95152	348	2.73593	2.73593
Gliocladium roseum	339	8.30279	119	1.6169	1.6169
Glomus mossae	396	0.47018	396	0.46726	0.46726
Macrophomina phaseolina	188	5.41028	327	6.37722	5.41028
Penicillium variabile	346	2.95152	71	1.90235	1.90235
Plectosphaerella	343	8.30279	138	4.93304	4.93304
Plectosphaerella cucumerina	342	8.30279	138	4.93304	4.93304
Pyrenochaeta (Phoma) lycopersici	189	5.41028	328	6.37722	5.41028
Verticillium nigrescens	344	2.95152	84	1.99956	1.99956