Project Title	Protected tomato: monitoring rhizosphere micro-organisms to improve understanding and management of root diseases
Project number:	PC 281
Project leader:	Dr Tim O'Neill ADAS UK Ltd, Boxworth, Cambs
Report:	Annual, April 2010
Previous reports:	February 2009
Key staff:	Ms Sarah Deery and Dr Matt Dickinson, University of Nottingham Mr Chris Dyer and Ms Sue Cahill, ADAS
Location of project:	ADAS Boxworth, Cambs University of Nottingham, Sutton Bonington Campus, Leics. Commercial nurseries (Cambs, Isle of Wight, Kent, Norfolk, Worcs)
Project coordinators:	Dr Phil Morley, Wight Salads Group Mr John Overvoorde, Delfland Nursery Mr Paul Simmonds, Cornerways Nursery
Date project commenced:	1 April 2008
Date completion due:	31 March 2011
Key words:	Tomato, root pathogens, saprotrophs, T-RFLP, isolation

© 2010 Agriculture and Horticulture Development Board

Disclaimer

Whilst reports issued under the auspices of the HDC are prepared from the best available information, neither the authors nor the HDC can accept any responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of results.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use except where the crop or situation is the subject of an off-label extension of use.

Before using all pesticides and herbicides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the HDC office (hdc@hdc.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

Horticultural Development Company
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel: 0247 669 2051

© 2010 Agriculture and Horticulture Development Board

No part of this publication may be reproduced in any form or by any means without prior permission from the HDC.

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.

Dr T M O'Neill
Principal Research Scientist
ADAS UK Ltd

Signature ..... Date .....

Report authorised by:

Mr J Clarke
Science and Business Development Manager
ADAS UK Ltd

Signature ..... Date .....

© 2010 Agriculture and Horticulture Development Board

CONTENTS

© 2010 Agriculture and Horticulture Development Board

GROWER SUMMARY

Headline

Over 90 fungal and 120 bacterial species have been indentified on tomato roots, including 12 fungi likely to be pathogenic to tomato.

Background and expected deliverables

Root-infecting fungi are commonly found in tomato and occasionally result in severe disease. Non-pathogenic fungi and bacteria are also common in the root environment and the presence and levels of these 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 method is also able to detect non-culturable micro-organisms. The project aims to use T-RFLP to investigate the occurrence and relative levels of major pathogenic (e.g. species of *Pythium, Phytophthora, Fusarium, Thielaviopsis*) and non-pathogenic micro-organisms (e.g. species of *Penicillium, Pseudomonas, Trichoderma*) associated with roots of tomato crops in various substrates.

The expected deliverables from this project are:

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

Summary of the project and main conclusions

<u>Effect of growing medium and crop age on microbial populations on tomato roots</u>

In 2009 the microbial populations associated with tomato roots were determined by T-RFLP analysis on 90 samples. 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 (soon after planting, at first pick and in early August). Samples of irrigation solution drainage water were also examined. Most of the plants from which roots were sampled remained alive and

© 2010 Agriculture and Horticulture Development Board

healthy at the end of cropping but a few were dead or affected by Verticillium wilt, a *Fusarium* species or vascular staining. Black dot (*Colletotrichum coccodes*) and black root rot (*Thielaviopsis basicola*) were observed quite commonly on roots, especially of plants grown in NFT solution.

T-RFLP analysis indicated the presence of 92-100 fungi and 127-161 bacteria associated with the sampled tomato roots. Sixty-six fungi and more than 100 bacteria were identified using a computer programme (Fragsort) to match DNA fragment lengths against a database of reference fungi and bacteria that the authors have created based on published DNA sequence information. Individual fungal species that each comprised more than 0.2% of the total population were:

- *Colletotrichum coccodes*
- *Cylindrocarpon destructans* (cause of brown root rot)
- *Fusarium* sp. (a potential cause of wilt and root rots)
- *Gigaspora rosea* (an endomycorrhizal fungus)
- *Lycoperdon* sp.
- *Macrophomina phaseolina* (cause of charcoal root rot).

G. rosea was present at the greatest levels. Mycorrhizal fungi such as *G. rosea* have been reported to increase resistance to root diseases in some crops.

From the Fragsort output of probable fungal identities, 12 were considered potential pathogens of tomato (Table 1). The potential pathogens found most frequently were *Plectosphaerella cucumerina,* which was detected in seven of the 10 crops, *C. coccodes* in five and a *Fusarium* species (potentially *F. oxysporum*) in three. *P. cucumerina* is a common inhabitant of arable soils and has been associated with root, stem and leaf damage of tomato seedlings and other hosts; the asexual stage of this fungus is *Fusarium tabacinum*. Around 45% of fungal DNA fragments and 28% of bacterial DNA fragments were not identified (i.e. there was no match in the database).

© 2010 Agriculture and Horticulture Development Board

Table 1: Potential fungal pathogens found during routine sampling of tomato roots from 10 crops - 2009

		Growing medium and crop reference number									
Fragment length	Probable identity [a]	RW		Soil		NFT		Coir		WF	
(bp)		1	2	3	4	5	6	7	8	9	10
H73	*Fusarium* sp.		✓		✓					✓	
H81	*Humicola fuscoatra*							✓			
H84	*Verticillium nigrescens*				✓						
H126	*Alternaria solani*							✓			✓
H138	*Plectosphaerella cucumerina* [b]	✓*	✓*		✓	✓*	✓*		✓	✓*	
H153	*Colletotrichum coccodes*	✓*		✓*			✓*	✓	✓*		
H293	*Phytophthora capsici* [c]									✓	
H294	*Phytophthora cinnamomi* [c]			✓							
H311	*Phytophthora cinnamomi (contig2)* [c]										✓
H327	*Macrophomina phaseolina*	✓									
H328	*Pyrenochaeta lycopersici*				✓*				✓		
H593	*Pythium oligandrum*			✓							

RW – rockwool; WF – woodfibre; * relatively abundant

[a] Determined by Fragsort computer programme which seeks matches between fragment lengths generated during sample tests with entries in a fungal and bacterial database. The accuracy of identifications is dependant on the accuracy of the entries lodged in the database.
[b] This fungus has a *Fusarium* asexual stage (*Fusarium tabacinum*).
[c] Confirmation of identification is required, *P. capsici* is not indigenous in the UK, *P. cinnamomi* has previously been reported as a pathogen of tomato in the USA, not in the UK.

© 2010 Agriculture and Horticulture Development Board

Many of the fungi identified by Fragsort as occurring on roots of UK tomato crops were expected, but some were unexpected (e.g. *Phytophthora capsici*). Further work is required to determine the accuracy of these unexpected findings indicated by Fragsort. Microbial population diversity on roots was examined using Simpson's Diversity Index, a measure that takes account of both species richness and relative abundance. The value ranges between 0 (poor diversity) and 1 (high diversity). Fungal population diversity increased significantly with plant age (from 0.6 to 0.7), whereas bacterial population diversity was higher (0.8) and unaffected by plant age or other factors. Growth medium had a large effect on fungal population diversity, being least in NFT (0.61) and greatest in soil (0.68). Fungal diversity increased progressively with time in rockwool, NFT and coir crops, but decreased with time in soil crops (Figure 1).

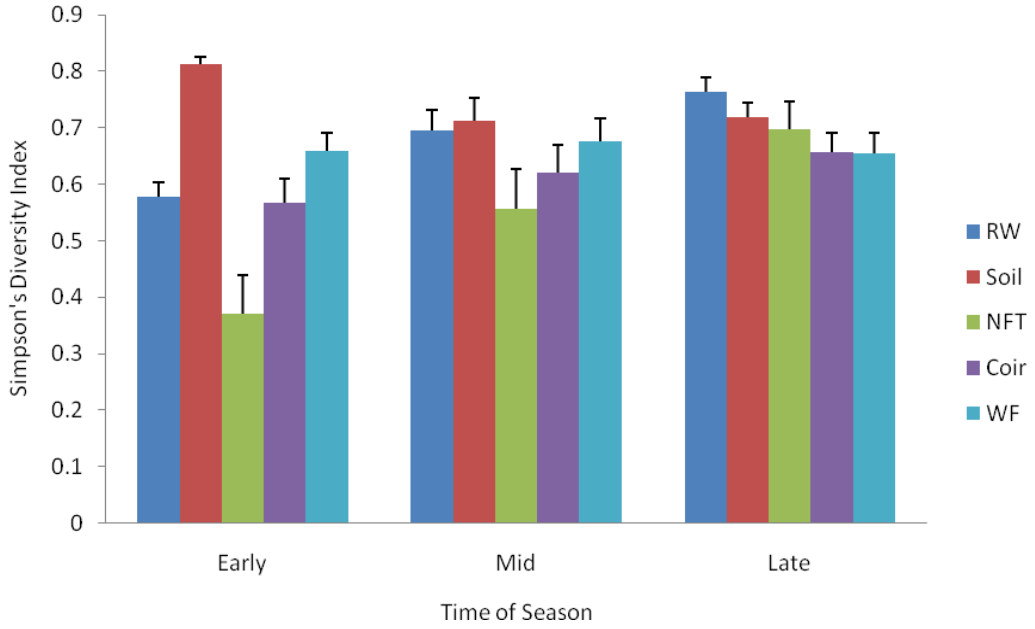


Figure 1: Effect of sample time (plant age) and growth medium on fungal diversity calculated using the Simpson's Diversity equation for all 10 crops at 3 sampling times in 2009.

Principal component analysis was used to examine which DNA fragments (micro-organisms) contributed most to variation between samples. For the fungal relative abundance data, the first three principal components, comprising seven fragments, explained 56% of the variation. Three of the fragments were identified, as *Plectosphaerella cucumerina* (x 2) and *Gigaspora rosea*, the others were not identified (Table 2).

© 2010 Agriculture and Horticulture Development Board

Table 2: DNA fragments making a significant contribution to variation in fungal populations between samples, and their possible identities

Fragment length (bp)	Possible identify	Comment
A205	Unidentified (U1)	Present in soil crops
A343	*Plectosphaerella cucumerina*	Present in RW, soil and NFT crops
A385	Unidentified (U2)	Dominant in NFT; and present late in season
A386	Unidentified (U3)	Dominant in RW, soil, coir, WF and early season
H138	*Plectosphaerella cucumerina*	Present in RW, soil and NFT crops
H174	Unidentified (U4)	Present in soil crops
H343	*Gigaspora rosea*	Present in RW, soil and NFT crops

For the bacterial relative abundance data, the first three principal components, comprising 8 DNA fragments, explained 48% of total variation between samples. Three of these were identified using the Fragsort database: *Bacillus* sp., *Rhodobacter sphaeroides* and *Pasteurella multocida*. None of these are plant pathogens. Examination of bacterial population structure by this method suggests that it is influenced by plant age and growth medium, even though no difference was detected in diversity using Simpson's Diversity Index.

Association of crop observations and root microbial populations

The results of T-RFLP monitoring during crop production were in accordance with crop observations for *F. oxysporum* and *P. lycopersici* in crop 4 and *C. coccodes* in crop 6. *P. cucumerina* has a *Fusarium* stage and possibly the *Fusarium* sp. observed in crops 6 and 8 was *P. cucumerina*. A greater number of likely fungal pathogens were found associated with roots during crop production by T-RFLP analysis than were observed on the same plants either during sample collection or at the end of cropping (Table 3). However, although several plants died from Verticillium wilt in coir crop 7, this was not detected by T-RFLP, possibly because of the late development of the disease as no symptoms were visible at the time of the third root sampling. T-RFLP was also unable to confirm the presence of *Thielaviopsis basicola* in the two NFT crops probably because the enzymes used result in TRFs of the same or similar fragment length to *C. coccodes* which was also present in these samples.

© 2010 Agriculture and Horticulture Development Board

Table 3: Occurrence of possible fungal pathogens found associated with tomato roots as determined by T-RFLP analysis of routine root samples from 10 crops and microscope examination of symptomatic tissues during and at end of cropping – 2009

Possible pathogen	RW			Soil			NFT			Coir			WF	
	1	2		3	4		5	6		7	8		9	10
Alternaria solani										T				T
Colletotrichum coccodes	T			T			M	TM		T	T			
Fusarium sp.		T		M	TM			M			M		T	M
Humicola fuscoatra										T				
Macrophomina phaseolina	T													
Phytophthora sp. (1)													T	
Phytophthora sp. (2)		T												
Phytophthora sp. (3)														T
Plectosphaerella cucumerina	T	T			T		T	T			T		T	
Pyrenochaeta lycopersici				M	TM						T			
Pythium oligandrum				T										
Verticillium sp.					T					M	M			M
Thielaviopsis basicola							M	M						

T - determined by T-RFLP; M - determined by microscopy;

RW – rockwool; WF – Woodfibre

Plant and root health was assessed at the end of cropping for the nine monitored plants in each of the 10 crops. Plant health and root health scores for each crop were compared with microbial diversity on roots. Our hypothesis was that plants with a low microbial diversity on roots would be more susceptible to root disease and vice-versa. Although the crops differed in end-of-season root rot scores and in fungal diversity, there was no obvious association of root rot with fungal diversity indices (Figure 2). This 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.

Current work is being undertaken in two related projects at the University of Nottingham to refine the T-RFLP test, and to develop additional tests that can be used to build upon the progress made using T-RFLP. Pyrosequencing is being used in collaboration with Fera, York to identify the fungal and bacterial species that are responsible for the 'unidentified' peaks present in T-RFLP traces. This will enable us to provide more definitive identifications to be made of all the organisms present in samples. In a second University of Nottingham/Fera collaborative project on tomato roots, an array-based

© 2010 Agriculture and Horticulture Development Board

system is being developed in which DNA sequences from fungi and oomycetes known to be associated with tomato roots (pathogens and non-pathogens) are being arrayed. These arrays will then be probed with DNA extracted from the tomato root samples to provide additional information on the species present and more reliable quantification data. Together these related projects will provide additional data and resources to aid predictions of root health in commercial crops and to provide a basis for the use of amendments and practices to improve root health.

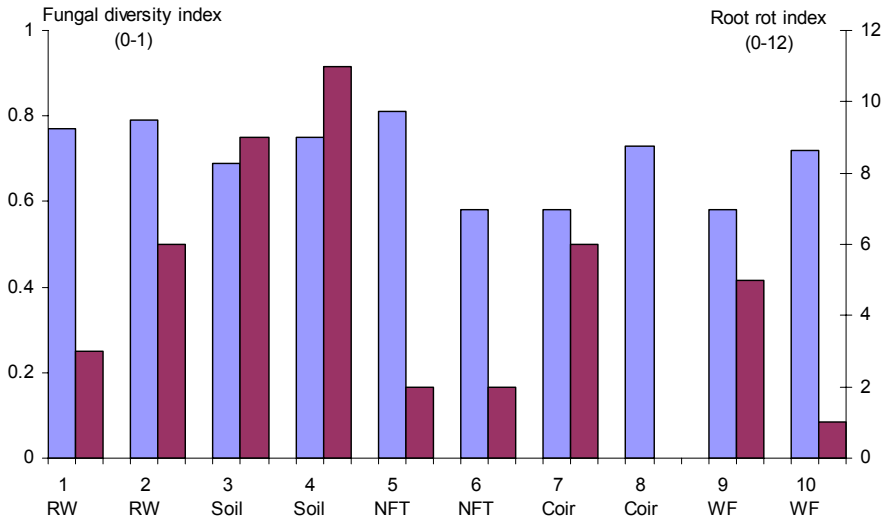


Figure 2: Fungal diversity index in August and root rot index at the end of cropping in 10 tomato crops – 2009 (RW = rockwool; WF = woodfibre)

Effect of some specific factors on microbial populations associated with tomato roots

Paired root samples were collected from seven crops to examine the effect of some specific factors on microbial populations associated with tomato roots. Factors examined were the occurrence of root mat symptoms and root browning, each compared with healthy plants. Additionally, development of microbial population over time was examined by T-RFLP analysis of a seed batch and of young plants grown from the seed, both in propagation and following establishment on the production nursery.

Agrobacterium bv. 1 strains were not found in plants with root mat symptoms. In a comparison of affected and unaffected roots from adjacent plants in a rockwool crop, both fungal and bacterial species richness was greater in plants with root mat symptoms than those without. Possibly this is a result of secondary colonisation by micro-organisms due to release of growth substrates from colonised tissue. The fungi found at relatively high levels on affected roots were *P. cucumerina* and a *Phytophthora* species. The bacteria *Flavobacterium* spp. and *Bacillus* spp. were found associated with healthy

© 2010 Agriculture and Horticulture Development Board

roots. Certain *Bacillus* spp. are reported to be associated with root health and disease suppression.

In the comparison of brown roots and healthy roots from adjacent plants in a rockwool crop, fungal and bacterial species richness was greater in the brown roots, possibly a reflection of secondary colonisation. *Pythium ultimum* was confirmed at a relatively high level on the brown roots. *Pythium* spp. was also confirmed in the brown roots by microscope observation and by culturing from the roots.

In the comparison of micro-organisms on seed, and plants grown from the seed in a propagation house and on a production nursery, fungal species richness increased from seed to propagation house and then remained at the higher level on the production nursery. Bacterial species richness increased from seed to propagation house. No plant pathogens were detected on the seed. *Plectrosphaerella cucumerina* was detected in the propagation house and on the production nursery and a *Fusarium* sp. was detected on the nursery; the majority of fragments making a significant contribution to the populations at each stage in production were not identified.

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

A replicated experiment was done to determine the effect of drenches with Companion® (*Bacillus subtilis* GB03)and Trianum-P (*Trichoderma harzianum* T-22), and addition of *Dendrobaena* earthworms, on microbial populations associated with roots of soil-grown cv. Claree and occurrence of fusarium crown and root rot. Soil was drench-inoculated *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) spores after the first application of the potential control treatments. A second application of Companion and Trianum-P was applied one week later. Plants were grown for 16 weeks after potting in a greenhouse and yield was recorded.

No symptoms of fusarium crown and root rot developed in the experiment. Inoculation with FORL did not significantly affect fruit yield or root extent. Neither Companion or Trianum-P drench or earthworm amendment affected fruit yield. The addition of earthworms to soil resulted in poor, pale green growth, until potassium nitrate feeding was started. The effect of soil amendments on root microbial populations as determined by T-RFLP analysis on root samples will be presented in the Final report.

© 2010 Agriculture and Horticulture Development Board

Financial benefits

Root-infecting fungi are commonly found on tomato plants grown in soil, substrate and NFT crops and occasionally cause severe disease. On individual nurseries, root disease may result in widespread plant wilting and necessitate early crop removal. Yield loss due to root dieback associated with minor root pathogens is also possible. Estimates of yield loss to root diseases have not been reported. 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

None at present.

© 2010 Agriculture and Horticulture Development Board

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 better to 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

© 2010 Agriculture and Horticulture Development Board

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 the objectives are:

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

© 2010 Agriculture and Horticulture Development Board

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 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 examination of microbial populations on roots can be used to help predict the risk of root disease in a crop.

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. Although it was not possible to use a common variety at all sites, the range of varieties used was kept to a minimum. Standard or large fruit varieties were selected. 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.

© 2010 Agriculture and Horticulture Development Board

Table 1.1: Details of tomato crops monitored in 2009

Growing medium and site code	Variety	Date planted	Dates sampled			Final assessment
			Early	First pick	Main	
Rockwool						
1.	Roterno	Mid Dec	6 Jan	4 Mar	11 Aug	26 Oct
2.	Encore	Mid Dec	7 Jan	21 Apr	25 Sep	12 Nov
Soil						
3.	Sunstream[a]	End Feb	15 Apr	23 Jun	12 Aug	11 Nov
4.	Roterno[a]	End Mar	4 Mar	13 May	12 Aug	11 Nov
NFT						
5.	Aranka	Mid Dec	12 Jan	24 Mar	12 Aug	29 Oct
6.	Aranka	End Dec	22 Jan	7 Apr	5 Aug	29 Oct
Coir						
7.	Encore	Mid Jan	5 Feb	23 Mar	10 Aug	27 Oct
8.	Dometica	Mid Jan	3 Mar	20 Apr	24 Sep	17 Nov
Woodfibre						
9.	Roterno	Mid Dec	28 Jan	4 Mar	11 Aug	26 Oct
10.	Dometica	Mid Dec	3 Mar	20 Apr	24 Sep	17 Nov

[a] On Beaufort 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 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 (nil, slight, moderate or severe), and root

© 2010 Agriculture and Horticulture Development Board

appearance. 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 plant 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

Each root sample was cut into three sub-samples to provide nine microbial population profiles per nursery growing medium per sample time. Root samples (≥100 mg) were roughly chopped using a sterile scalpel blade. Samples were placed in a 2.0 mL tube (graduated skirted NAT) (Starlab, Ahrensburg, Germany) containing 10 acid washed glass beads (Sigma-Aldrich, Haverhill, UK). Root tissue was disrupted by vigorous shaking in Fastprep (QBiogene, Cambridge, UK) for 3 cycles of 45 seconds at 6.5 m s^{-1}. Total microbial DNA was extracted using Qiagen DNeasy Plant Mini Kit (Qiagen, Crawley, UK), following the manufacturers protocol. In instances where there was PCR inhibition, extracts were cleaned using PVPP (Cullen and Hirsch 1998).

Amplification of microbial communities

One µl of DNA extract was used in PCR amplification of the 23S ribosomal subunit for bacteria and the ITS-2 region for fungi. Primers used to amplify the 23S rRNA gene have been previously published (Anthony e*t al*. 2000). The 23S reverse primer (23Srev*) was fluorescently labeled with D4 Beckman dye (Sigma Proligo). The fungal primers targeted the 5.8S ribosomal subunit (5' GCATCGATGAAGAACGCAGC 3': 5.8s for) and from the 5' end of 28S ribosomal subunit (Ranjard *et al*. 2000). The fungal reverse primer (FITSrev1*) was fluorescently labeled with D3 Beckman dye (Sigma Proligo). Both fluorescent labels are suitable for analysis on CEQ8000 fragment analysis system (Beckman-Coulter, High Wycombe, UK). Amplifications were performed in 25 µl reactions containing 12.5 µl of 2xPCR master mix (Promega, Southampton, UK), 10.5 µl of sterile distilled water and 0.5 pmol of each primer in a Techne Progene thermal cycler. PCR conditions for 23s: 94^{o}C for 2 min, followed by 30 cycles of 94^{o}C for 0.5 min, 59^{o}C for 1 min and 72^{o}C for 1 min, completed with a final extension step of 72^{o}C for 10 min. Amplification of ITS2 was the same as for 23s, but with an annealing temperature of 53 ^{o}C. Amplification of ribosomal regions was confirmed by running PCR products a 1% agarose gel for 1hr at 90V and then viewing under UV light.

© 2010 Agriculture and Horticulture Development Board

Restriction digest

Two restriction enzymes were used compared with one in Year 1, in an attempt to increase the resolution of closely related organisms. PCR products were digested with: *Mse*I (T/TAA) and *Hae*III (GG/CC) or *Hae*III (GG/CC) AluI (AG/CT) (New England Biolabs, Hitchin, UK) for bacteria and fungi respectively. Five µl of PCR product was used in a 10 µl reaction volume containing 1U of restriction enzyme. Digests were incubated at 37^{o}C for 2 hours followed by denaturation of enzymes by heating to 80^{o}C for 20 min.

Terminal Restriction Fragment Length Polymorphism (T-RFLP)

Digests were mixed at a 2:1 ratio of fungal and bacterial digests respectively. Three µl of the digest mix were loaded into a 96 well plate with each well containing 38.5 µl of GenomeLab sample loading solution and 0.5 µl of GenomeLab size standard-600 (Beckman-Coulter). The Samples were overlaid with mineral oil and separated on a CEQ 8000 DNA analysis system (Beckman-Coulter). Fragments between 60 and 700bp were considered for analysis, with a dye signal greater than 100 fluorescent. All shoulder peaks, associated with base pair addition through the use of PCR amplification, were removed from analysis.

Cloning

PCR products were purified with the QIAquick PCR purification kit (Qiagen) and quantified on a Nanodrop. Approximately 50 nanograms of PCR product insert was used for cloning reactions using the pGEM cloning kit pGEM-T Easy Vector Systems (Promega), following the recommendations of the manufacturer. Insert-containing colonies were amplified using M13For and M13Rev, purified and then sequenced.

Sequencing

Purified PCR products were sequenced on a CEQ 8000 GeXP Genetic Analysis System (Beckman Coulter). Sequences were edited and BLASTED using Chromas version 2.33. Results were recorded and compared with confirmed and theoretical T-RFLP fragment lengths.

Cultures

Cultures were acquired from various sources, including ADAS and Fera (Appendix I). 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.

© 2010 Agriculture and Horticulture Development Board

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 (Sharma, 1995).

© 2010 Agriculture and Horticulture Development Board

Results and discussion

Fungi identified on tomato roots

Table 1.2 shows all probable fungal organisms identified by Fragsort from all samples collected in 2009, showing the mean relative abundance in all samples (%). From this table it is clear that cutting with an extra enzyme does increase the resolution of fungal peaks, for example; *Aspergillus flavus*, *Aspergillus fumigatus* and *Humicola fuscoatra* all have a TRF of 81bp when cut with *Hae*III, but separate out at 340, 339 and 188bp when cut with *Alu*I respectively.

However, the use of another enzyme does not resolve all closely related organisms, as can be seen from looking at *Fusarium oxysporum* and *Fusarium culmorum* which all have a TRF length of 73bp when cut with *Hae*II and 120bp when cut with *Alu*I.

The use of a third restriction enzyme could perhaps help solve this problem. Alvarado and Manjon (2009) suggest that for optimal results from T-RFLP analysis of fungal ITS, a set of at least three restriction enzymes is required. On the other hand, specific primers could be designed from NCBI sequence entries for the common tomato rhizosphere organisms that are difficult to separate with the two current restriction enzyme and could potentially produce a more definitive and cost effective result.

Many of the fungi identified by Fragsort as occurring on roots of UK tomato crops are ones we would expect to find. These include *Aspergillus* spp., *Colletotrichum coccodes, Cylindrocarpon destructans, Fusarium oxysporum, Gliocladum* spp. *Humicola fuscoatra, Mucor* sp., *Penicillium* spp., *Plectosphaerella cucumerina*, *Pythium* sp, *Trichoderma viride* and *Verticillium nigrescens.*

Some of the fungi identified by Fragsort as occurring on the roots of tomato crops are unexpected, notably *Phytophthora capsici* (considered non-indigenous), *Phytophthora cinnamomi* (not listed as a pathogen of tomato in the UK), and *Rhynchosporium secalis* (a pathogen of barley leaves). These may be true results and add to our knowledge or they may be mis-identifications due to errors in the database.

Plectosphaerella cucumerina is a common inhabitant of arable soils and has been associated with root, stem and leaf damage of tomato seedlings (Pascoe *et al*., 1984) and other hosts; the asexual stage of this fungus is *Fusarium tabacinum*. *Gigaspora rosea* is a vesicular-arbuscular mycorrhizal fungus. Colonisation of tomato roots by this

© 2010 Agriculture and Horticulture Development Board

fungus has been recorded previously in artificial inoculation studies (Scervino *et al*., 2005), and the fungus appeared to be common in commercial tomato crops in our work. The effect of colonisation of tomato roots by *G. rosea* on susceptibility to root diseases warrants further investigation.

Table 1.2: Fungal organisms identified in Fragsort from routine samples collected in 2009, showing TRF size when cut with *Alu*I and *Hae*III and relative abundance (%)

Organism name	*Alu*I TRF size	*Hae*III TRF size	Abundance (%)
Alternaria alternata, Alternaria tenuissima or *Cylindrocarpon* spp.	162	124	0.02
Alternaria solani	196	126	0.03
Aspergillus flavus	340	81	0.13
Aspergillus fumigatus	339	81	0.05
Aspergillus niger	340	82	0.04
Aspergillus sydowii	339	83	0.04
Aureobasidium pullulans	193	132	0.02
Calyptella campanula	81	370	0.08
Candida albicans	324	324	0.13
Cephalosporium spp.	196	75	0.03
Chaetomium bostrychodes	326	70	0.10
Cladosporium cladosporioides	323	323	0.06
Cladosporium herbarum	322	322	0.02
Colletotrichum acutatum	329	154	0.17
Colletotrichum coccodes	189	153	0.70
Colletotrichum fragariae	165	155	0.02
Cylindrocarpon destructans	119	75	0.24
Didymella spp.	192	331	0.01
Doratomyces sp.	95	72	0.02
Epicoccum nigrum	189	328	0.02
Epicoccum spp.	190	329	0.08
Fusarium culmorum, F. oxysporum, F. oxysporum f. sp. lycopersici, F. oxysporum f. sp. radis-lycopersici, F. redolens or *Fusarium* spp.	120	73	0.25
Gigaspora rosea	342	343	2.77
Gliocladium spp.	341	156	0.17
Glomus intraradices	378	378	0.15
Glomus mossae	396	396	0.03
Humicola fuscoatra	188	81	0.13
Leptosphaeria spp.	188	333	0.01
Lycoperdon spp.	83	384	0.23
Macrophomina phaseolina	188	327	0.42
Microdochium nivale	165	339	0.02
Mucor meihei	93	69	0.01
Paecilomyces lilacinus	334	183	0.02
Penicillium digitatum	338	82	0.04

© 2010 Agriculture and Horticulture Development Board

Organism name	*Alu*I TRF size	*Hae*III TRF size	Abundance (%)
Penicillium griseofulvum	340	82	0.04
Penicillium janthinellum	341	82	0.04
Penicillium lividum	335	79	0.06
Penicillium purpurogenum	329	329	0.08
Penicillium thomii	332	81	0.13
Penicillium verrucosum	338	81	0.05
Petriella spp.	155	70	0.02
Phoma destructiva	191	330	0.01
Phytophthora cactorum or *P. citricola*	158	589	0.03
Phytophthora capsici	161	293	0.02
Phytophthora cinnamomi	161	294	0.02
Phytophthora cinnamomi (contig2)	161	311	0.02
Plectosphaerella	343	138	0.07
Plectosphaerella cucumerina	342	138	0.07
Pyrenochaeta (Phoma) lycopersici	189	328	0.02
Pythium oligandrum	161	593	0.02
Rhizopus microsporus	164	183	0.02
Rhizopus oryzae	361	361	0.04
Rhynchosporium secalis	182	147	0.09
Sclerotinia cepivorum or *S. sclerotiorum*	174	318	0.06
Trichoderma viride	349	155	0.03
Trichurus spp.	95	72	0.02
Verticillium nigrescens	344	84	0.04

The effect of growing medium on fungi associated with tomato roots as identified by Fragsort is shown in Table 1.3.

Table 1.3: Identified fungi in five growing media and their relative abundance (%) – 2009

Possible fungus	Rockwool	Soil	NFT	Coir	WF
Alternaria solani					*
Aspergillus flavus				*	
Aspergillus niger		0.12		*	
Aspergillus sydowii		0.12			
Candida albicans	0.59		0.14	*	*
Cephalosporium spp.					*
Chaetomium bostrychodes		0.24		*	*
Cladosporium cladosporioides	0.04		0.14	*	*
Cladosporium herbarum	0.04		0.14	*	*
Colletotrichum acutatum	2.44	0.15	1.85		
Colletotrichum coccodes	0.71	0.41	0.45		
Colletotrichum fragariae			0.03		
Cylindrocarpon destructans					*
Didymella spp.			0.48		
Doratomyces sp.					*
Epicoccum nigrum - isolate M7	0.59	0.04			
Epicoccum spp		0.04	0.45		
Fusarium culmorum	0.15				*
Fusarium oxysporum	0.15				*

© 2010 Agriculture and Horticulture Development Board

Fusarium oxysporum f. sp. lycopersici	0.15				*
Fusarium oxysporum f. sp. radis-lycopersici	0.15				*
Fusarium redolens	0.15				*
Gigaspora rosea	3.91	0.19	5.83	*	*
Gliocladium spp.				*	
Glomus intraradices		0.71			
Glomus mossae	0.08				
Leptosphaeria spp.			0.45		
Lycoperdon spp.				*	
Lycoperdon spp. *2*				*	
Macrophomina phaseolina	0.59	1.72			
Paecilomyces lilacinus	0.59	0.11		*	
Penicillium digitatum		0.12			
Penicillium griseofulvum		0.12		*	
Penicillium janthinellum		0.12		*	
Penicillium lividum					*
Penicillium purpurogenum		0.04	0.48		*
Penicillium thomii				*	
Petriella spp.					*
Plectosphaerella	7.02	0.17	5.83	*	*
Plectosphaerella cucumerina	7.02	0.17	5.83	*	*
Pyrenochaeta (Phoma) lycopersici	0.59	0.04			
Pythium oligandrum		0.10			
Rhizopus microsporus			0.03		
Rhizopus oryzae	0.15				
Rhynchosporium secalis		0.05			
Tomato	42.48	33.39	15.12	*	*
Trichoderma viride		0.15			
Trichurus spp.					*
Verticillium nigrescens		0.12			

* Present but not quantified using the same enzyme as used for rockwool, soil and NFT.

It is important to note that the output of the Fragsort program is limited to the size and accuracy of the database it compares T-RFLP profiles to. From looking at Table 1.4 it is apparent that the current fungal database is insufficient in size and cannot account for 52% of the TRFs produced from digestion with *Alu*I and cannot identify 40% of the peaks when cut with *Hae*III. To overcome this issue, another search of the NCBI database for fungal ITS2 regions is required. In addition, PCR products from routine sampling containing unknown peaks could be cloned and sequenced. The sequence data could then be compared to sequences of previously published data to identify the organism and then TRFs added to the database.

Table 1.4: Fungal fragments cut with *Alu*I and *Hae*III unidentified by Fragsort

Fungal Fragments	*Alu*I	*Hae*III
Number of Fragments:	92	100
Number of Unidentified Fragments:	47	40
Percent of Unidentified Fragments:	51.09%	40%

© 2010 Agriculture and Horticulture Development Board

From the Fragsort output of probable fungal identities, out of the 100 fragment entries there were 12 possible pathogens identified (Figure 1.1; Table 1.5) from pathogens previously reported on tomato (Year 1 report). To confirm the presence of these organisms, samples containing the relevant peaks should be cloned and sequenced. In addition, it will be interesting to compare these results with the end of season root health assessment.

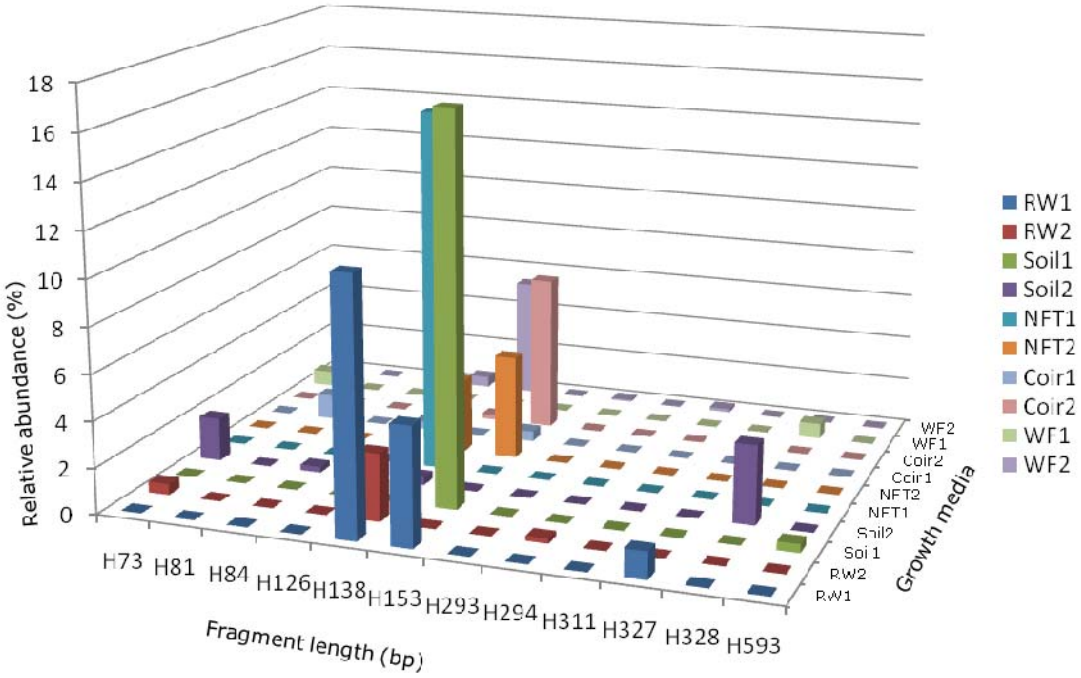


Figure 1.1: Mean relative abundance of 9 T-RFLP profiles for each of the 10 crops showing potential fungal organisms and their fragment length when cut with *Hae*III (*RW = rockwool; WF = woodfibre*). Table 1.5 shows identity of potential organisms.

Table 1.5: Potential pathogens found in routine sample from 10 sites and their fragment length when cut with *Hae*III

Fragment length (bp)	Potential pathogens	Site no. with peak present	Growing media
H73	*Fusarium* sp.	2;4;9	RW, Soil, WF
H81	*Humicola fuscoatra*	7	Coir
H84	*Verticillium nigrescens*	4	Soil
H126	*Alternaria solani*	7;10	Coir, WF
H138	*Plectosphaerella cucumerina*	1;2;4;5;6;8;9	All
H153	*Colletotrichum coccodes*	1;3;6;7;8	RW, Soil, NFT, Coir
H293	*Phytophthora capsici*	9	WF
H294	*Phytophthora cinnamomi*	2	RW
H311	*Phytophthora cinnamomi (contig2)*	10	WF
H327	*Macrophomina phaseolina*	1	RW
H328	*Pyrenochaeta lycopersici*	4;8	Soil, Coir
H593	*Pythium oligandrum*	3	Soil

For site numbers see Table 1.1; whilst Fragsort suggests these identifications, it is possible that some of these may represent other organisms and/or non-pathogenic isolates of the fungal species.

© 2010 Agriculture and Horticulture Development Board

Fragsort also highlighted the fact the tomato plant DNA peak took up over 40% of the total fungal community DNA composition. This is quite problematic in that with the tomato having such dominance, lower levels of fungi present may get missed with this analysis. To overcome this problem, other methods of DNA extraction will have to be examined to attempt to reduce the plant peak.

Bacteria identified on tomato roots

The bacteria identified by Fragsort on routine samples collected in 2009 are listed in Appendix 1. Fragsort produced 347 possible bacterial identifications for 90-118 DNA fragments. This indicates a greater degree of uncertainty over the accuracy of bacterial identifications compared with fungal identifications (63 possible identifications for 45-60 DNA fragments). Two of the possible bacterial species identified by Fragsort are known pathogens of tomato; *Corynebacterium michiganensis* pv*. michiganensis* and *Ralstonia solanacearum.* These findings are very unexpected and should be treated as tentative. In both instances, Fragsort produced several alternative possible identifications for these fragment lengths. Further work is required to determine the true results.

As with the fungal database, Fragsort highlighted that the bacterial database is also insufficient in size (Table 1.6) with 29% of TRFs unidentified when cut with *Hae*III and 27% when cut with *Mse*I. To increase the percentage of fragments identified by Fragsort, all samples containing unknown TRFs should be cloned and sequenced, with the theoretical TRFs added to the database.

Table 1.6: Bacterial fragments cut with *Hae*III and *Mse*I unidentified by Fragsort

Bacterial Fragments	*Hae*III	*Mse*I
Number of Fragments:	127	161
Number of Unidentified Fragments:	37	43
Percent of Unidentified Fragments:	29.13%	26.71%

Microbial population diversity

Simpson's Diversity Index was used. This is a composite equation that accounts for both evenness (a measure of the relative abundance of the different species) and richness (number of species per sample) characteristics of community assemblages and is a robust measure for statistical analyses (Magurran 2004). The value of this index ranges between 0 and 1, the greater the value, the greater the sample diversity. Determination of Simpson's diversity index from T-RFLP relative abundance data

© 2010 Agriculture and Horticulture Development Board

provides an insight into the effect of sample time (plant age) and tomato growth substrate on bacterial and fungal diversity.

From fungal relative abundance data of all routine sampling, Simpson's index values show that plant age (sample time) had a significant effect ($F_{2,225}$ = 21.81, P= < 0.001) (Table 1.7) on fungal diversity, with diversity (on average for all crops) increasing progressively over the three sampling times (Figure 1.2). This result indicates that the fungal community increases with species richness and evenness as the plant develops. Since production of root-released chemicals can vary during plant and root development (Swinnen *et al*., 1994), it might be expected that microbial communities in the rhizosphere could be influenced by the developmental stage and age of a plant. Such exudates can exert a selective microbial stimulation (Buir and Caesar, 1984) which varies in function of time due to plant age.

Growth medium also had a significant effect on fungal diversity ($F_{4,\ 225}$ = 21.81, P= < 0.001) (Figure 1.3) with soil having the greatest fungal diversity (0.68) and NFT with the least (0.61). It is a well known that microbial community is affected by numerous environmental variables, including growth medium (Head *et al*., 1998).

It is perhaps not surprising to find that NFT had the lowest fungal diversity as it is well documented that the nutrient solutions in such systems lack the microbial diversity, such conditions can allow soil-borne pathogens to grow and spread quickly (Paulitz, 1997). It has also been known for a long time that soil diversity has a huge array of organisms that co-exist in many cases without competitive exclusion (Heywood 1995).

© 2010 Agriculture and Horticulture Development Board

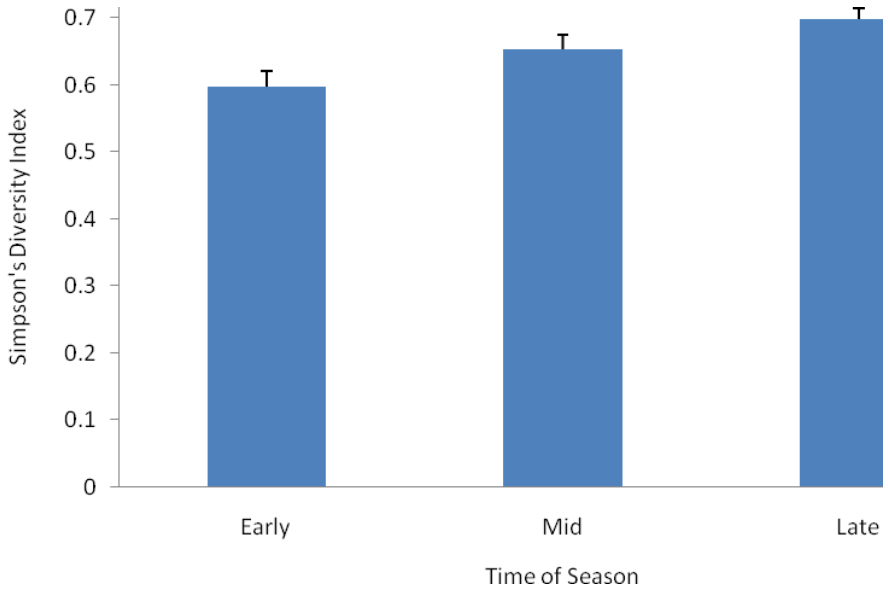


Figure 1.2: The effect of sample time (plant age) on fungal diversity calculated using the Simpson's Diversity equations for all 10 crops at 3 sampling times in 2009.

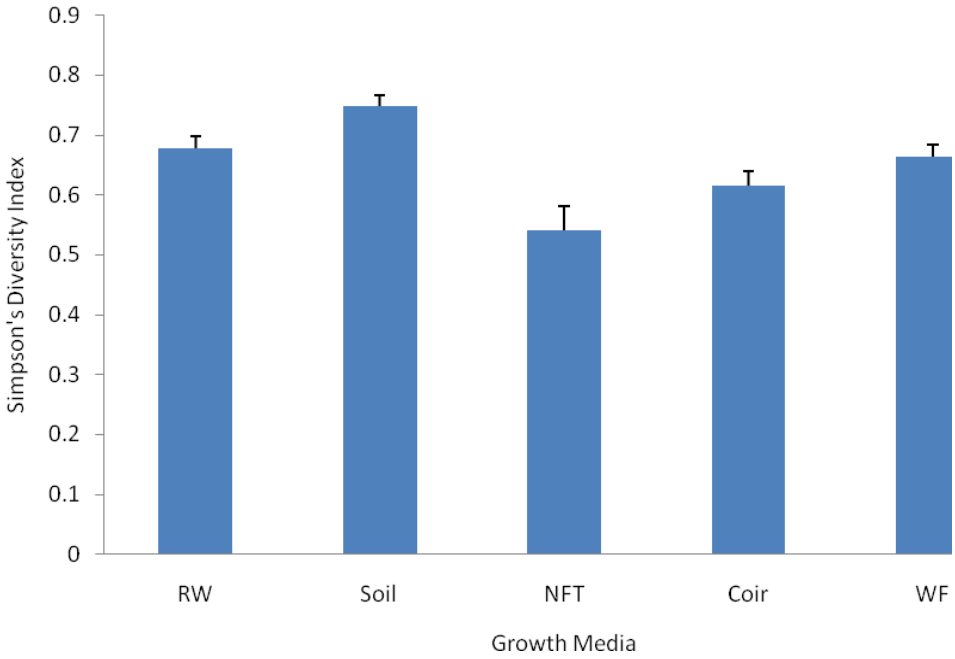


Figure 1.3: The effect of growth medium on fungal diversity calculated using the Simpson's Diversity equation for all 10 crops in 2009.

In addition to the above effects on fungal diversity, results from an ANOVA conducted on Simpson's Diversity Index results show that the effect of growth medium x plant age

© 2010 Agriculture and Horticulture Development Board

interaction (Figure 1.4) also had a significant effect on fungal diversity (Table 1.7) ($F_{8,225}$ = 4.01, P= < 0.001). Figure 1.4 shows that Rockwool, NFT and Coir fungal diversity increases progressively with time, whereas this is the opposite for soil, where fungal diversity decreases progressively with time. Woodfibre fungal diversity appears to change little with time.

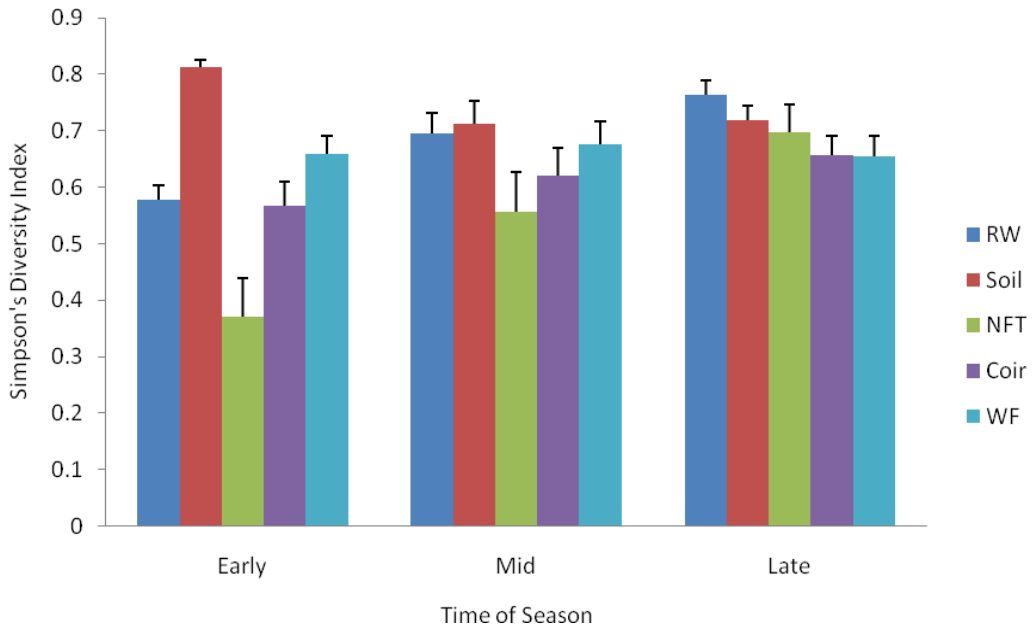


Figure 1.4: Effect of sample time (plant age) and growth medium on fungal diversity calculated using the Simpson's Diversity equation for all 10 crops at 3 sampling times in 2009

Table 1.7 ANOVA results for the interaction between growth medium and plant age (sample time) on fungal diversity calculated using the Simpson's Diversity Index

	Degrees of Freedom	Sum of Squares	Mean Squares	F-Ratio	Probability
Growth Medium	4	1.28074	0.32019	10.13	<.001
Sample Time	2	0.45199	0.226	7.15	<.001
Growth Medium x Sample Time	8	1.01515	0.12689	4.01	<.001
Residual	255	8.06047	0.03161		
Total	269	10.80836	0.04018		

Bacterial diversity was greater than fungal diversity in all average sample times (Figure 1.5), in all growth media (Figure 1.6) and at all crop growth stages within each growth medium (Figure 1.7). However, neither factor (sample time or growth medium) had a significant effect on bacterial diversity. In addition to this, the interaction between plant

© 2010 Agriculture and Horticulture Development Board

age and sample time also has no significant effect on bacterial diversity ($F_{8,225}$ = 0.054, P= < 0.001) (see Table 1.8 for interactions).

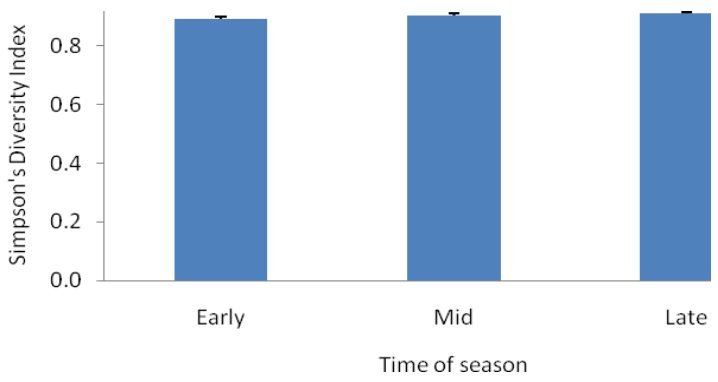


Figure 1.5: The effect of sample time (plant age) on bacterial diversity calculated using the Simpson's Diversity equations for all 10 crops at 3 sampling times in 2009.

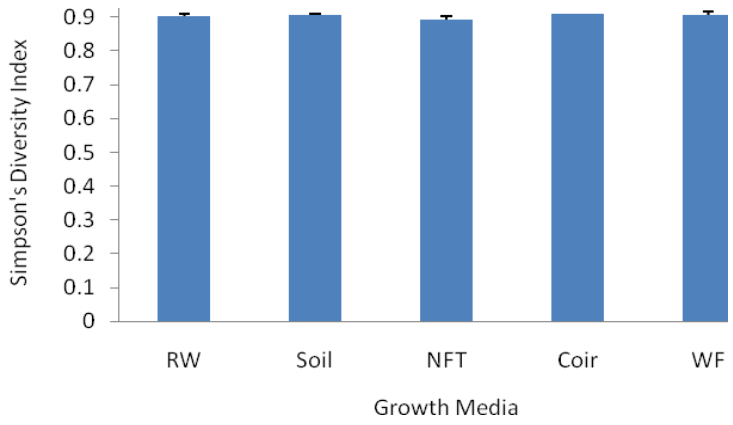


Figure 1.6: The effect of growth medium on bacterial diversity calculated using the Simpson's Diversity equation for all 10 crops in 2009.

© 2010 Agriculture and Horticulture Development Board

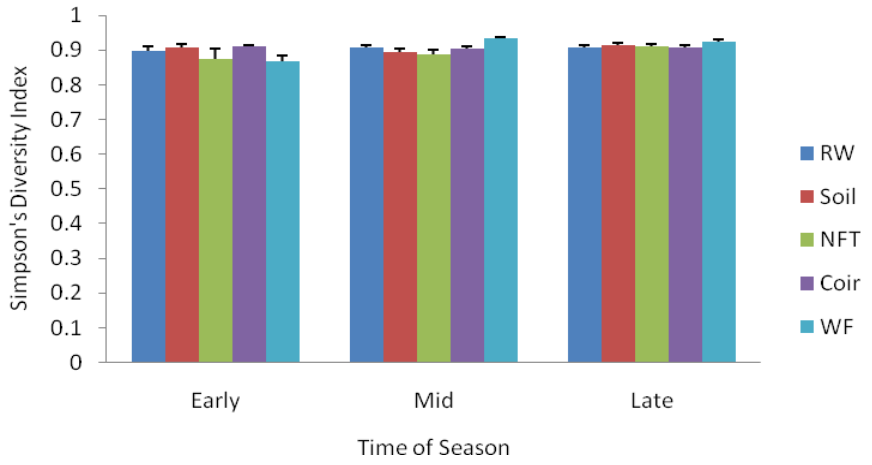


Figure 1.7: Effect of sample time (plant age) and growth medium on bacterial diversity calculated using the Simpson's Diversity equation for all 10 crops at 3 sampling times in 2009.

Table 1.8: ANOVA results for the interaction between growth medium and plant age (sample time) on bacterial diversity calculated using the Simpson's Diversity Index.

	Degrees of Freedom	Sum of Squares	Mean Squares	F-Ratio	Probability
Growth Medium	4	0.010616	0.002654	0.99	0.412
Sample Time	2	0.02076	0.01038	3.89	0.022
Growth Medium x Sample Time	8	0.041488	0.005186	1.94	0.054
Residual	255	0.681241	0.002672		
Total	269	0.754105	0.002803		

The effect of variety on fungal and bacterial diversity using Simpson's Diversity Index is shown in Figures 1.8 – 1.9.

© 2010 Agriculture and Horticulture Development Board

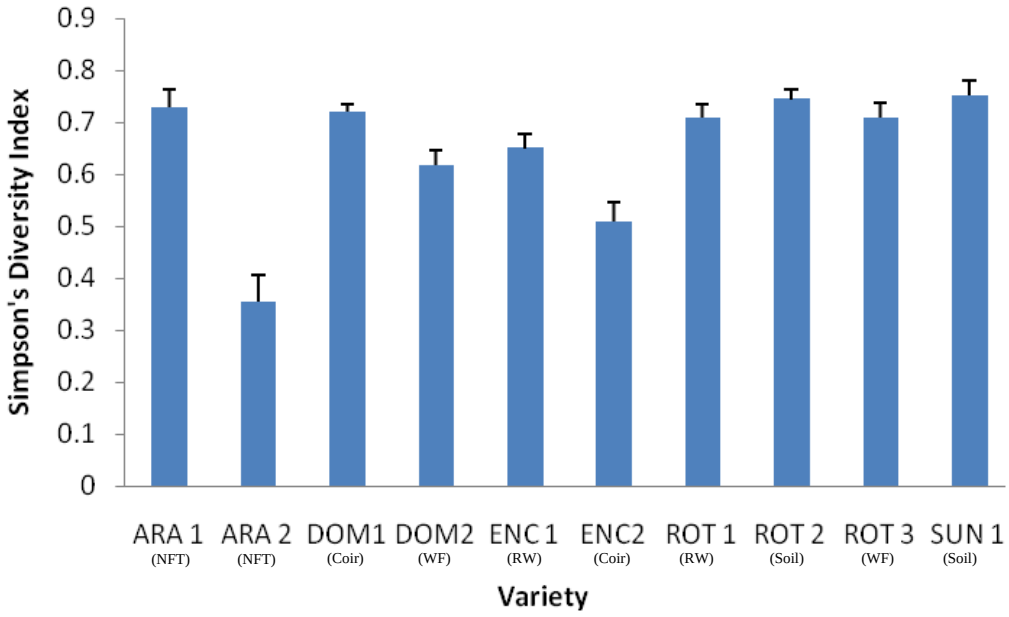


Figure 1.8: Tomato variety effect on fungal diversity calculated using the Simpson's Diversity Equation. Ara-Aranka; Dom-Dometica; Enc-Encore; Rot-Roterno; Sun-Sunstream

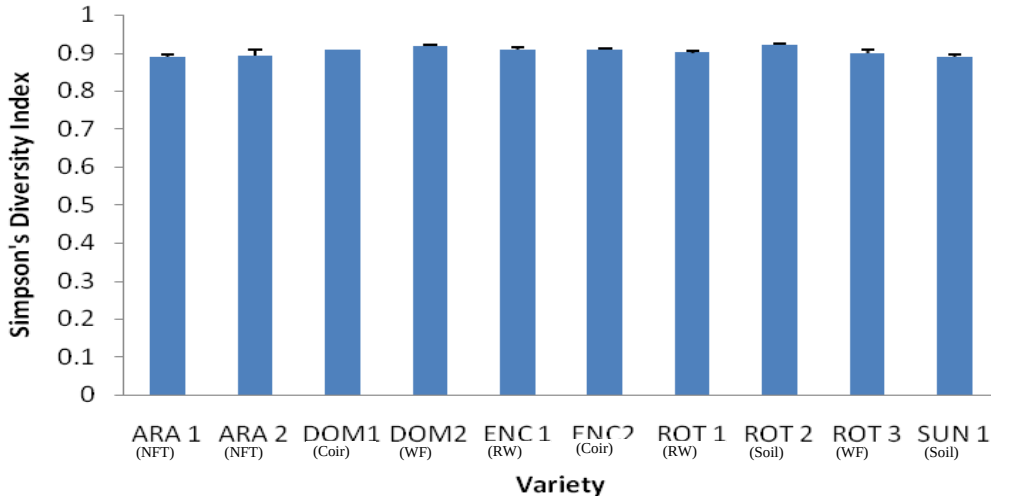


Figure 1.9: Tomato variety effect on bacterial diversity calculated using the Simpson's Diversity equation. See Fig. 1.8 for variety names.

PCA Analysis of microbial population diversity

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

© 2010 Agriculture and Horticulture Development Board

Fungal community

For the fungal relative abundance data set, principal component 1 (PC1) accounted for 31.96 % of the total variation within all routine samples, PC2 accounted for 14.26 % and PC3 accounted for 10.19 % (Table 1.9). These first three principal components explained a total variation of 56.41%, providing good coverage of the data.

Table 1.9: Results of principal component analysis of fungal T-RFLP relative abundance for all samples taken in 2009. (*Note: eigenvalues are the sum of the squared factor loads for any given factor and can be thought of as the amount of variance for that factor*)

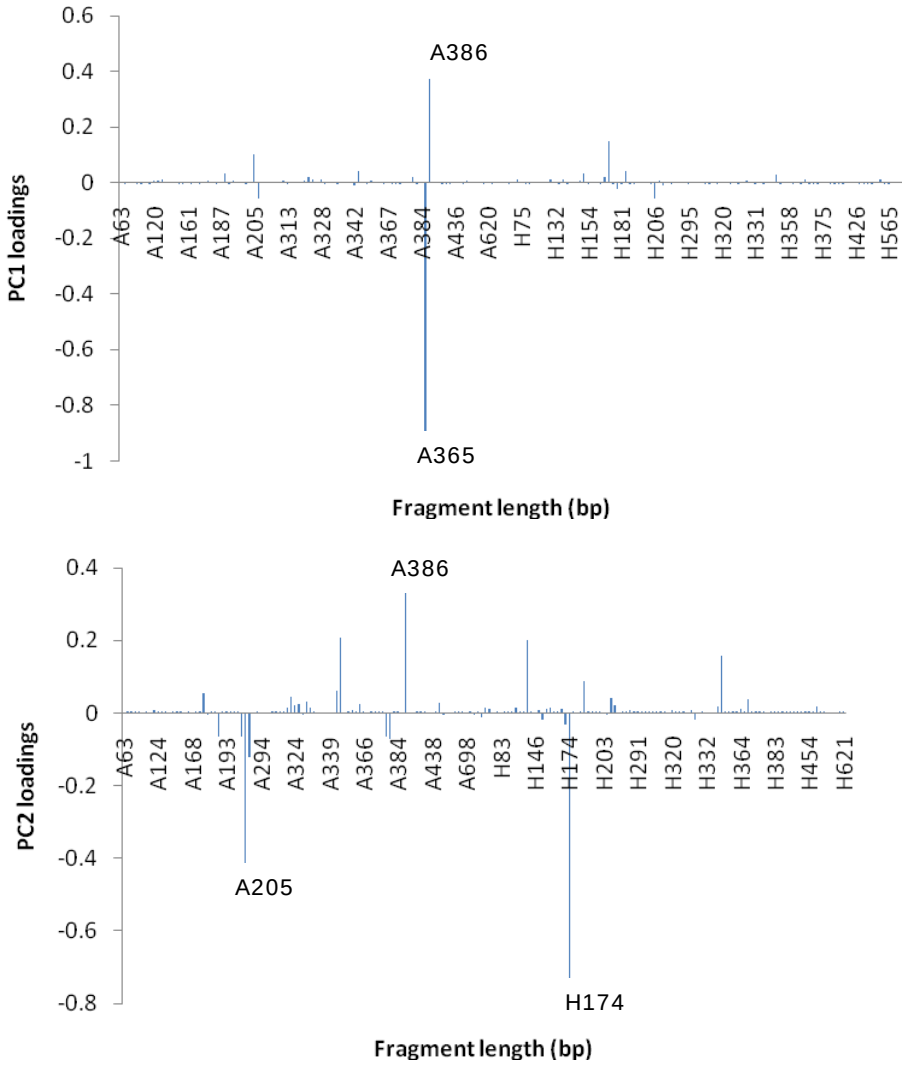
	PC 1	PC 2	PC 3
Eigenvalues	755.6	337.1	240.8
% Variance	31.96	14.26	10.19
Cumulative (%)	31.96	46.22	56.41

Factor loadings describe which fragments contribute the most variation in the principal component analysis. Factor loading values were analyzed for all fragments to ascertain which were making a significant contribution to PC1, PC2 and PC3. The fragments with the highest loading values in each PC axis were identified and any fragments with PC loading values > ±0.25 were classed as making a significant contribution to the principal component (Pio *et al*., 1996). Therefore, PC1 can be described as the presence and absence of two fragments, PC2 is determined by three fragments and PC3 by four (Figure 1.10). For example PC1 shows that when a sample profile contains the fragment A385bp it would have a negative PC1 score, whereas, if the PC1 score was positive the fragment A386bp would be present (code A = when cut with *Alu*I). Due to the similar size of these two fragments when cut with AluI, it is reasonable to assume that these two organisms could be closely related.

It would also be reasonable to predict that fragments A343 and H343 (code A = when cut with *Alu*I*;* H = when cut with *Hae*III) significantly contributing to PC3 loadings (Figure 1.10) are the same organism uncut with either enzyme. However, this prediction does not correlate with output from Fragsort (Table 1.2) which suggests that A343 is a *Plectospharella sp*. and H343 is *Gigaspora rosea* (meaning that no organism in the current database is left uncut with both *Alu*I and *Hae*III, with total fragment length of 343). Nonetheless, the fact that there was no organism present in the database giving a peak at 343 when uncut by either enzyme does not rule out the chance that these peaks could be from the same organism; it could merely be that the organism is not in the current database.

© 2010 Agriculture and Horticulture Development Board

To confirm whether peaks A385 and A386 are closely related, as well as whether peaks A343 and H343 are in fact the same organism, PCR products containing high levels of these peaks (preferably the majority of total community population) should be cloned and sequenced. The resulting sequences should be analyzed against previously published sequences on NCBI website to confirm their identity.



© 2010 Agriculture and Horticulture Development Board

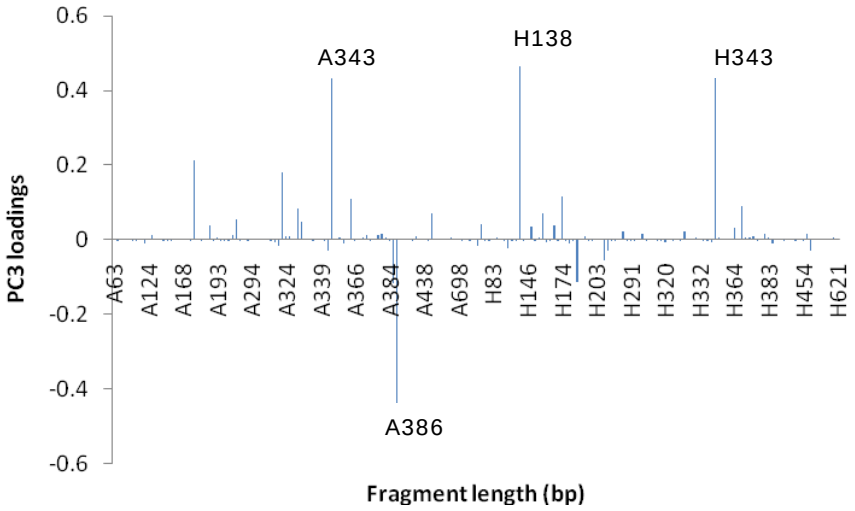


Figure 1.10: PC loadings for principal components 1, 2 and 3 from fungal relative abundance data from routine sampling in 2009. (*Fragment length codes A = when cut with AluI; H = when cut with HaeIII).*

Fragments making a significant contribution to PC1, PC2 and PC3 were correlated with Fragsort output data (Table 1.2) to give possible identities of the organisms responsible for these groupings (Table 1.10). Only three out of seven significant fragments could be given possible identities, once again highlighting the insufficient size of the current fungal ITS2 database. The most effective way to overcome this difficulty and to confirm the 'possible identity' of significant peaks is to clone and sequence from PCR products containing relevant peaks.

Table 1.10: Fragments making a significant contribution to PC1, PC2 and PC3, and their possible identities (*Fragment length codes A = when cut with AluI; H = when cut with HaeIII)*

Fragment length (bp)	Possible Identity
A205	Unidentified (U1)
A343	*Plectosphaerella cucumerina.*
A385	Unidentified (U2)
A386	Unidentified (U3)
H138	*Plectosphaerella cucumerina*
H174	Unidentified (U4)
H343	*Gigaspora rosea*

Figure 1.11 depicts an ordination plot of PC1 scores versus PC2 scores from the five different growth medium at all the sampling times (crop growth stages). An ANOVA of PC1 scores showed that growth medium ($F_{9,240}$ = 21.81, P= < 0.001) and sample time ($F_{2,240}$ = 18.30 , P= < 0.001) had a significant effect on PC1. Fungal DNA profiles from

© 2010 Agriculture and Horticulture Development Board

rockwool, soil, coir and woodfibre all have positive PC1 scores, as can be seen on the ordination plot (Figure 1.11), suggesting the dominance of fragment A386. NFT has a negative PC1 score signifying the presence of fragment A385. As mentioned above, season time also had a significant effect on PC1 with early and mid season samples having negative PC1 scores (early=-1.67; mid=-1.24), whilst late had a positive PC1 score (1.9). This suggests the presence of fragment A 386 at the start of the season and A385 late on in the growing season (N.B see Table 1.11 for all interactions within the dataset).

PC2 grouped with growing medium ($F_{9,240}$ = 17.83, P= < 0.001). PC2 scores for rockwool, NFT , coir and woodfibre all have positive PC1 scores, as can be seen on the ordination plot (Figure 1.11) suggesting the presence of A386. Soil on the other hand has a negative PC2 score, which implies the presence of A205 and H174.

Results from ANOVA of PC3 scores show that growing medium ($F_{9,240}$ = 4.36, P= < 0.001) had a significant effect on PC3 scores, with rockwool, soil and NFT having positive PC3 scores (1.31; 3.55; 9.82 respectively) suggesting the presence of A343 (*Plectosphaerella cucumerina*), H138 (*P. cucumerina)* and H343 *(Gigaspora rosea)*. Coir and woodfibre have negative PC3 scores (-7.44; -7.23), implying the presence A386.

With *P. cucumerina* being a previously reported pathogen, it is perhaps unusual that is it in such abundance; present in 7 out of the 10 crops (Table 1.5) with a total relative abundance of 0.07% of the total population (Table 1.2). However, *P. cucumerina* is a well known constituent of the rhizosphere of many plants, including tomato and will only become a problem under appropriate conditions (Uecker, 1993).

© 2010 Agriculture and Horticulture Development Board

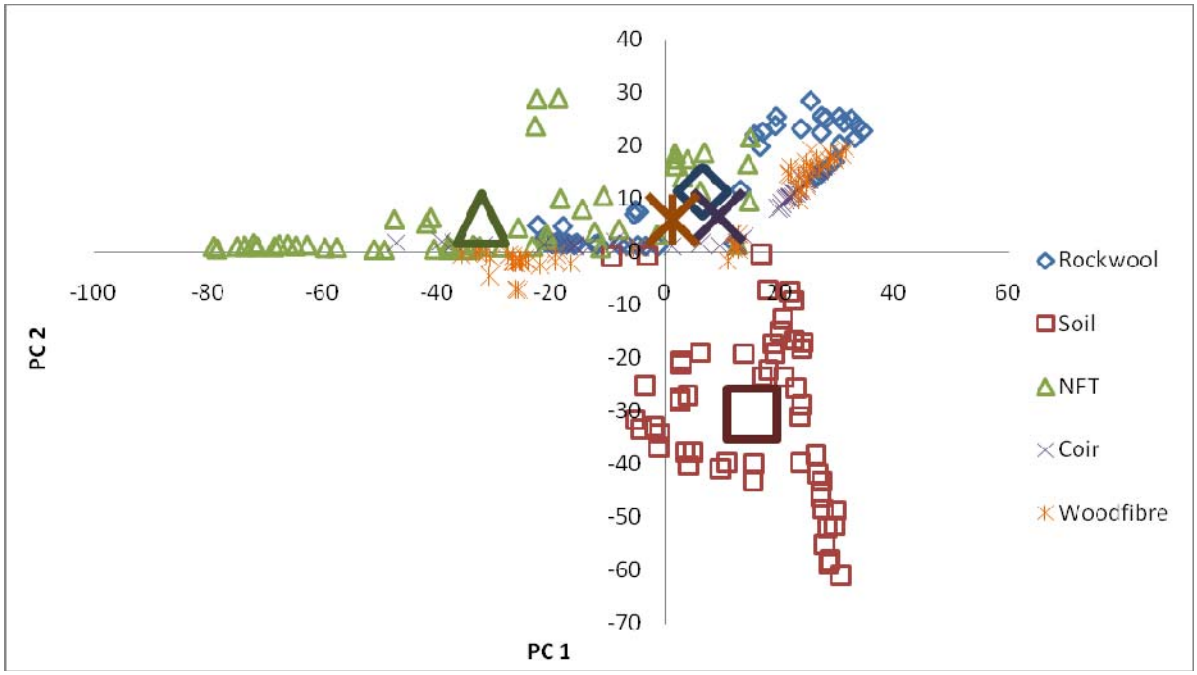


Figure 1.11: Ordination plot of PC1 versus PC2 scores for fungal relative abundance data from routine sampling of 10 crops from 5 different growth media.

Note: Large symbols represent mean PC score centroids.

Table 1.11: Results from ANOVA for PC1, PC2 and PC3 scores originating from fungal data from T-RFLP profiles

Source of Variation	DF	F	P
PC1			
Growing Medium	9	21.81	< 0.001
Sample Time	2	18.30	< 0.001
Growing Medium x Sample Time	18	18.74	< 0.001
Residual	240		
PC2			
Growing Medium	9	17.83	< 0.001
Sample Time	2	0.62	0.538
Residual	240		
PC3			
Growing Medium	9	3.46	< 0.001
Sample Time	2	1.55	0.215
Residual	240		

Bacterial community

For the bacterial relative abundance data set, principal component 1 (PC1) accounted for 21.66 % of the total variation within all routine samples, PC2 accounted for 15.37 % and PC3 accounted for 10.55 % (Table 1.12). These first three principal components explained a total variation of 47.58%, providing reasonable coverage of the data.

© 2010 Agriculture and Horticulture Development Board

Table 1.12: Results of principal component analysis of bacterial T-RFLP relative abundance for all samples taken in 2009

	PC 1	PC 2	PC 3
Eigenvalues	465.1	330	226.5
% Variance	21.66	15.37	10.55
Cumulative (%)	21.66	37.03	47.58

Factor loading values were analyzed for all fragments to ascertain which were making a significant contribution to PC1, PC2 and PC3. The fragments with the highest loading values in each PC axis were identified and any fragments with PC loading values > ±0.25 were classed as making a significant contribution to the principal component (Pio *et al*., 1996). Therefore, PC1 can be described as the presence and absence of five fragments, PC2 is determined by three fragments and PC3 by four (Figure 1.12).

Fragments making a significant contribution to PC1, PC2 and PC3 were correlated with Fragsort output data (Appendix 1) to give possible identities of the organisms responsible for these groupings. Only three out of eight significant fragments could be given possible identities, once again highlighting the insufficient size of the current bacterial database (Table 1.13). The most effective way to overcome this difficulty and to confirm the 'possible identity' of significant peaks is to clone and sequence from PCR products containing relevant peaks.

© 2010 Agriculture and Horticulture Development Board

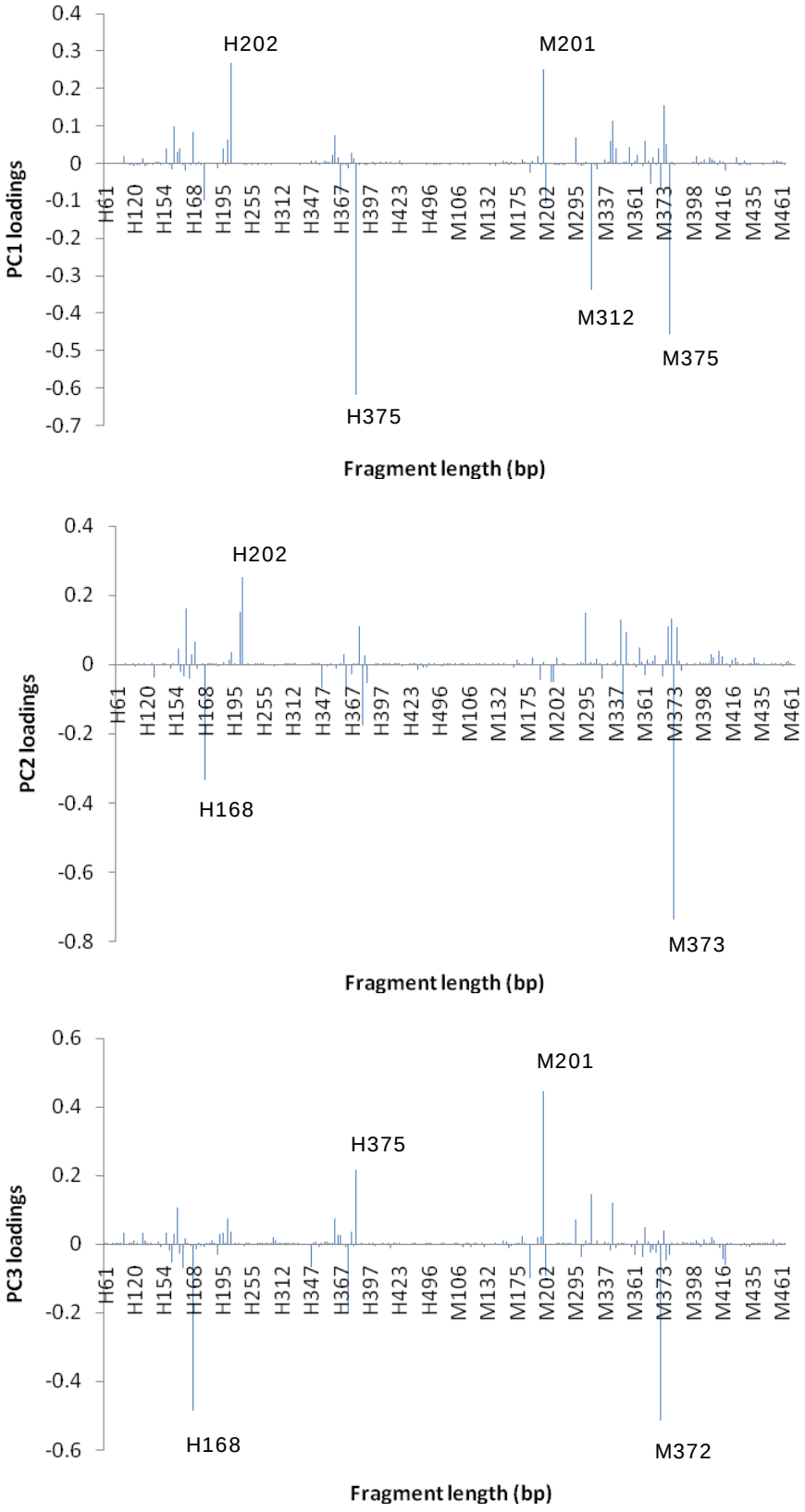


Figure 1.12: PC loadings for principal components 1, 2 and 3 from bacterial relative abundance data from routine sampling of 10 tomato crops.

© 2010 Agriculture and Horticulture Development Board

Table 1.13: Significant peaks contributing to PC1, PC2 and PC3 and their possible identity (*Fragment length codes M = when cut with MseI; H = when cut with HaeIII).*

Fragment length (bp)	Possible Identity
H168	*Bacillus* sp.
H202	Unidentified (U5)
H375	Unidentified (U6)
M201	Unidentified (U7)
M312	*Rhodobacter sphaeroides*
M372	Unidentified (U8)
M373	*Pasteurella multocida*
M375	Unidentified (U9)

Figure 1.13 depicts an ordination plot of PC1 scores versus PC2 scores from the five different growth medium at all the sampling times (crop growth stages). An ANOVA of PC1 scores showed that growth medium ($F_{9,240}$ = 83.68, P= < 0.001) had a significant effect on PC1. Bacterial DNA profiles from rockwool, soil and coir all have positive PC1 scores, as can be seen on the ordination plot, suggesting the presence of fragments H202 and M201. NFT and woodfibre have a negative PC1 score signifying the presence of fragments H375, M375 and M312 (*Rhodobacter sphaeroides)* (N.B see Table 1.14 for all interactions within the dataset).

Results from ANOVA of PC2 scores show that growing medium ($F_{9,240}$= 68.68, P= < 0.001) and season time ($F_{2,240}$= 4.46, P= < 0.001) have a significant effect on PC2. PC2 scores for NFT and coir have a positive PC1 score, as can be seen on the ordination plot (Figure 1.13) suggesting the presence of H202. Rockwool, soil and woodfibre have negative PC2 scores, which implies the presence of H168 (*Bacillus* sp.*)* and M373 *(Pasteurella multocida)*. Early season has a positive PC2 score (0.08) implying the presence of H202, whereas, mid and late season PC2 score are negative (-0.01; -0.06 respectively) suggesting the presence of H168 (*Bacillus* sp.*)* and M373 *(Pasteurella multocida)* becoming more dominant with time.

PC3 scores group by sample time ($F_{2,240}$ = 4.36, P= < 0.001), with early season samples having a positive PC3 score (0.12) suggesting the presence of H375 and M201. Mid and late season samples have a negative PC3 scores (-0.11; -0.01 respectively), once again implying the presence of H168 (*Bacillus* sp.) and M272.

These results suggest that bacterial community constituents are influenced by plant age and growth media despite no differences in diversity, as seen by Griffiths *et al*. (2001).

© 2010 Agriculture and Horticulture Development Board

Further information on the bacterial community in different growth media is given in Appendix 3.

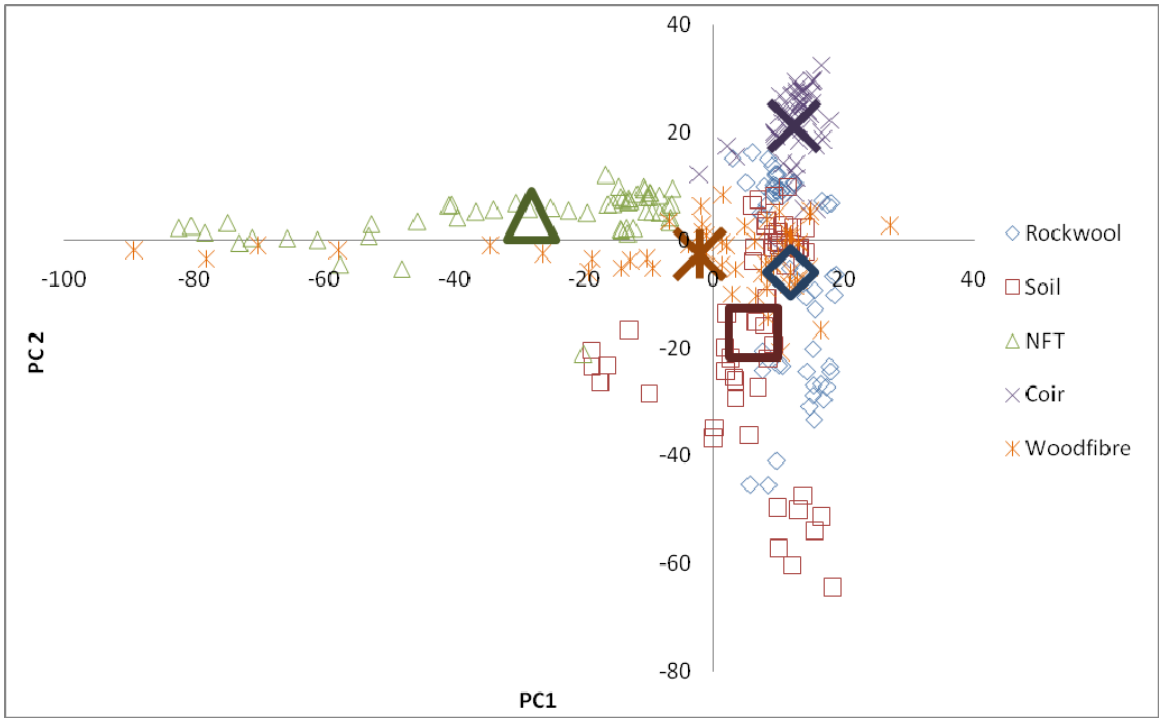


Figure 1.13: Ordination plot of PC1 versus PC2 scores for bacterial relative abundance data from routine sampling of 10 crops from 5 different growth media.

Note: Large symbols represent mean PC score centroids.

Table 1.14: Results from ANOVA for PC1, PC2 and PC3 scores originating from bacterial data from T-RFLP profiles

Source of Variation	DF	F	P
PC1			
Growing Medium	9	83.68	< 0.001
Sample Time	2	1.1	0.335
Total	269		
PC2			
Growing Medium	9	68.68	< 0.001
Sample Time	2	4.46	< 0.001
Growing Medium x Sample Time	18	9.83	< 0.001
Total	269		
PC3			
Growing Medium	9	0.94	0.49
Sample Time	2	31.32	< 0.001
Total	269		

© 2010 Agriculture and Horticulture Development Board

Plant and root health

Very few of the sampled plants showed symptoms of poor growth in August/September (Table 1.15). The exception was one soil crop (site 3) which showed wilting in August; one NFT crop (site 5) which showed leaf yellowing and discoloured roots in August; one coir crop (site 7) which showed leaf yellowing or wilting and discoloured roots in September.

At the end of cropping, all monitored plants in the two rockwool crops, the two NFT crops and one woodfibre crop were alive (Table 1.16). In contrast, four plants in each of the soil crops and four plants in one of the coir crops had died. A single plant had died in the second coir crop and the second woodfibre crop. *Verticillium* sp. was confirmed associated with dead plants in the coir and woodfibre crops. Cause of plant death in the soil crops was not determined but there was severe root decay, obvious vascular discolouration in the stem base of many plants and *Fusarium* sp. sporulation on the stem base of some plants. In all the crops where one or more monitored plants died, this occurred after mid-August.

Root blackening was obvious on the mass of fine roots in both NFT crops, and *Colletotrichum coccodes* and *Thielaviopsis basicola* was confirmed associated with these symptoms. A high density of fine roots was present in rockwool, coir and woodfibre slabs, and a good depth 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 are summarised in Table 1.17.

© 2010 Agriculture and Horticulture Development Board

Table 1.15: Summary of visual health of plants sampled for routine root monitoring – 2009

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	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	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	3	0	0	0	0	0
4.	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
5.	NFT	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	3	0	0	0	0	3	0
6.	NFT	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	3	0
		3	0	0	0	0	0	3	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	0	0
8.	Coir	1	0	0	0	0	0	0	0
		2	3	0	0	0	0	3	0
		3	0	3	0	0	0	3	0
9.	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
10.	Woodfibre	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	1	0

See Table 1.1 for sample dates.

© 2010 Agriculture and Horticulture Development Board

Table 1.16: Summary of root assessment at end of cropping in plants sampled for routine monitoring – 2009

Site	Growing medium	Number of plants (of 9):					Root mat	Mean severity (0-3)[a]:				Density of fine roots (0-3)
		Alive	Stem vascular browning	Verticillium sporing	Fusarium sporing	Root blackening		Stem vascular browning	Major roots decayed or brown	Minor roots decayed or brown	Corky roots present	
1.	Rockwool	9	9	0	0	0	0	2	1	1	0	3
2.	Rockwool	9	5	0	0	0	0	1	0	0	0	3
3.	Soil	5	6	0	3	0	0	2	2	3	2	1
4.	Soil	5	5*	0	1	0	0	3	3	3	2	1
5.	NFT	9	3	0	0	9	0	<1	0	2	0	2
6.	NFT	9	7	0	0	8	0	2	0	2	0	2
7.	Coir	5	5*	4	0	1	0	2	2	2	0	3
8.	Coir	8	8*	1	1	1	0	1	0	0	0	3
9.	Woodfibre	9	9	0	0	1	0	2	2	1	0	3
10.	Woodfibre	8	8*	1	1	0	0	2	0	1	0	3

*The other plants were dead; [a] 0 = nil: 1 =1-10%; 2 = 11-50%; 3 = >50%.

© 2010 Agriculture and Horticulture Development Board

Table 1.17: Summary of possible fungal pathogens found associated with nine plants during routine root monitoring or at the end of cropping by isolation onto agar and/or microscopy - 2009

Site	Growing medium	*Colletotrichum coccodes*	*Fusarium* spp.[a]	*Pyrenochaeta lycopersici*	*Thielaviopsis basicola*	*Verticillium* spp.[b]
1.	Rockwool	-	-	-	-	-
2.	Rockwool	-	-	-	-	-
3.	Soil	-	✓	✓	-	-
4.	Soil	-	✓	✓	-	-
5.	NFT	✓	-	-	✓	-
6.	NFT	✓	(✓)	-	✓	-
7.	Coir	-	-	-	-	✓
8.	Coir	-	✓	-	-	✓
9.	Woodfibre	-	-	-	-	-
10.	Woodfibre	-	✓	-	-	✓

[a] Probably *F. oxysporum*; [b] Probably *V. albo-atrum*

(✓) – on non-monitored wilting plants in the same row.

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 0-27; 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 1.18). 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 1.18)

There was no obvious association between either 'plant sickness' or 'root rot' scores and fungal diversity (Figures 1.14 and 1.15). This 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.

Bacterial diversity is not shown as this differed very little between crops or sample dates, ranging from 0.83 to 0.94.

© 2010 Agriculture and Horticulture Development Board

Table 1.18: Plant health and root health at the end of cropping in 10 tomato crops – 2009

Assessment	RW		Soil		NFT		Coir		WF	
	1	2	3	4	5	6	7	8	9	10
Incidence (of 9 plants)										
Number plants dead	0	0	4	4	0	0	4	1	0	1
Number with stem vascular browning	9	5	6	5	3	7	5	8	9	8
Number with visible stem *Verticillium*	0	0	0	0	0	0	4	1	0	1
Number with visible stem *Fusarium*	0	0	3	1	0	0	0	1	0	1
Severity (0-3)										
Major roots decayed/brown	1	0	2	3	0	0	2	0	2	0
Minor roots decayed/brown or black	1	0	3	3	2	2	2	0	1	1
Corky roots present	0	0	2	2	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 – 27)	9	5	14	13	3	7	14	10	9	10
Root rot score (2 x major root decay + no. corky + no. minor root decay) (0 – 12)	3	0	9	11	2	2	6	0	5	1

RW- rockwool; WF- Woodfibre.

© 2010 Agriculture and Horticulture Development Board

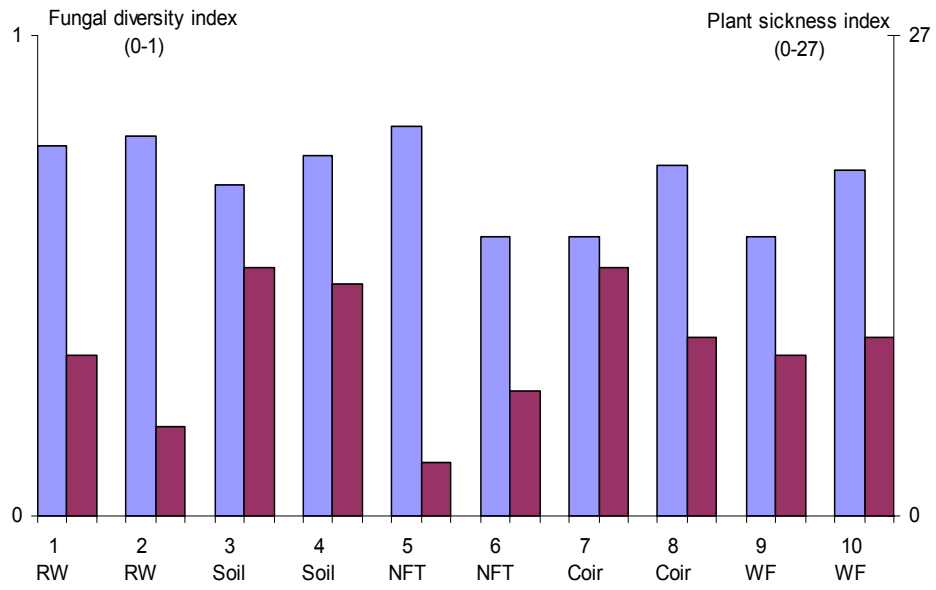


Figure 1.14: Fungal diversity index in August and 'plant sickness' at the end of cropping in 10 tomato crops – 2009

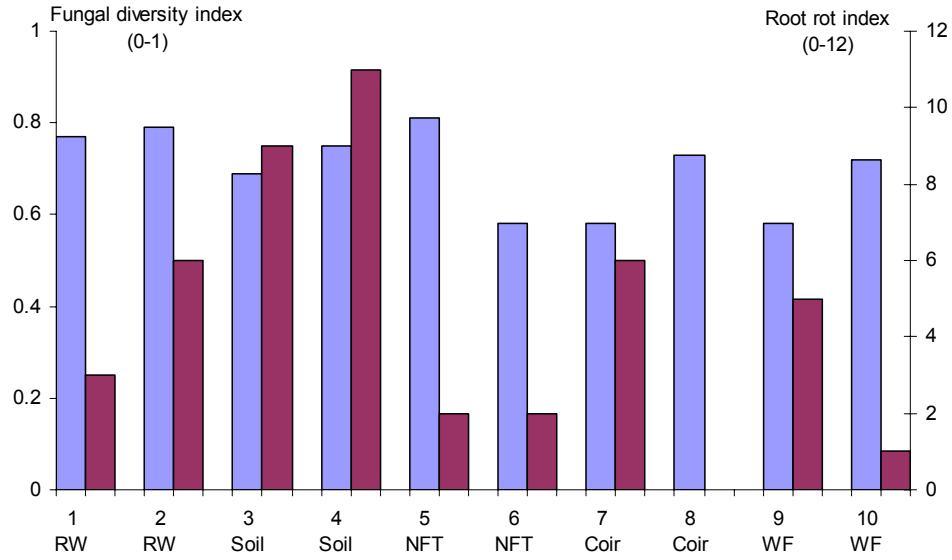


Figure 1.15: Fungal diversity index in August and 'root rot' and the end of cropping in 10 tomato crops - 2009

© 2010 Agriculture and Horticulture Development Board

Conclusions

Fragsort is a useful tool for rapid non-bias determination of TRF identity. The program gave 63 possible fungal identities and 347 possible bacterial identities for the routine sampling T-RFLP profiles. However, the benefit of this program is limited to the accuracy and size of the database it compares TRFs to. From Tables 1.3 and 1.5 it is clear that both the fungal and bacterial databases are insufficient in size, with Fragsort unable to identify 46% of fungal TRFs (on average) and 28% of the bacterial TRFs (on average). Re-analysis of previously reported sequences of 23S ribosomal region in bacteria and ITS2 region in fungi needs to take place to reduce this shortfall. In addition, samples with unidentified peaks need to be cloned and sequenced, compared to existing sequences for identity and added to the database.

Simpson's Diversity Index is a robust method for calculating population diversity; it accounts for both evenness and richness characteristics of a community. From conducting analysis of variance on the index output, it was found that plant age (sample time) ($F_{2,225}$ = 21.81, P= < 0.001), growth medium ($F_{4,225}$ = 21.81, P= < 0.001), as well as the growth medium x plant age interaction ($F_{8,225}$ = 4.01, P= < 0.001) had a significant effect on fungal diversity (Table 1.7). This indicates that the fungal community changes with species richness and evenness as the plant develops. Since production of root-released materials can vary during plant and root development (Swinnen *et al*., 1994), it might be expected that microbial communities in the rhizosphere could be influenced by the developmental stage and age of a plant. In addition, it is a well known that microbial community is affected by numerous environmental variables, including growth medium (Head *et al*., 1998).

There was no statistical significance between plant age and growth medium on bacterial diversity from the analysis of variance of the Simpson's Diversity output (Table 1.8). Levels of bacterial diversity appear to keep relatively constant throughout plant development and between media. Bacterial diversity was greater than fungal diversity in all average sample times (Figure 1.5), in all growth medium (Figure 1.6) and at all crop growth stages within each growth medium (Figure 1.7).

PCA provides a useful means to separate and group samples based on their community patterns. The first three principal components explained a total variation of 56.41% of fungal data and 47.58% of bacterial data. ANOVA results of principal components in correlation with ordination plots shows fungal and bacterial profiles were found to differ between sample time and between growth media. Results suggest that bacterial community constituents are influenced by plant age and growth media despite no differences in diversity, as seen by Griffiths *et al*. (2001). In addition,

© 2010 Agriculture and Horticulture Development Board

it would be useful to be able to identify all peaks statistically significantly contributing to PC1, PC2 and PC3 for all of the 10 crops.

To assess levels of which organisms contribute to root health, relative abundance data analysed by Simpson's Diversity Index and normalized data analyzed by PCA analysis for each of the 10 crops needs to be compared with the end of season root health assessment (Table 1.18).

© 2010 Agriculture and Horticulture Development Board

2. Effect of some specific factors on microbial populations associated with roots

Introduction

Various factors are likely to influence the populations of microorganisms 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. 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 microorganisms associated with tomato roots – 2009

Treatment comparison	Growing medium	Variety	Date sampled
1. Root mat present vs absent	Rockwool	Lucino	20 Apr 09
2. Brown roots vs white roots	Rockwool	-	6 Mar 09
3. Seed and propagation vs production nursery	Rockwool	DRC542	8 Dec 08 & 28 Jan 09
4. [a]Compete Plus monthly drench vs none	Ekofibre	Cheramy	25 May 09
5. New vs one year old coir slabs	Coir	Dometica	3 Mar 09
6. UV vs untreated NFT solution	NFT	Aranka	27 Jul 09
7. Roots from yellowing vs healthy crop	Soil	Roterno	7 May 09

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

[b] *Pythium* root rot confirmed in brown rots.

Root and solution samples

Samples were collected as described previously. For each paired comparison, three samples of each level (present/absent) were collected on one occasion. Three sub-samples from each were examined by T-RFLP (i.e. 9 T-RFLP profiles per factor).

© 2010 Agriculture and Horticulture Development Board

Results and discussion

2.1 Root mat versus healthy roots

Roots with and without root mat symptoms were sampled from the same row of rockwool grown tomato plants at the first sign of root mat disease symptoms. TRF peak number has been used as a measure of species richness in fungal and bacterial communities (Dunbar *et al*. 2000). Fungal species richness was higher in plants with root mat than in healthy plants ($F_{(1,16)}$ = 34.09, P = <0.001) (Figure 2.1). This was also true for bacterial species richness (Figure 2.2), however differences between plants with root mat symptoms and without were not significant ($F_{(1,16)}$ = 4.35, P = 0.045).

These findings do not generally correspond with current theory which indicates higher rhizosphere species richness being associated with healthy plants when compared with diseased plants (Filion *et al*., 2004). However, such findings are usually found at the onset of disease.

In this case, the increase in fungal species richness could be explained by the colonization of secondary microbes on diseased roots due to the release of utilizable growth substrates from infected tissues (Gardener and Weller, 2001). The potential pathogen *Plectosphaerella cucumerina* was identified from Fragsort output from samples with root mat symptoms, although the partitioning of substrates released from infected plant tissues among detrimental (e.g., pathogenic), neutral (e.g. saprophytic), and beneficial (e.g. mutualistic) microbial populations is not well understood. It is also important to note that an individual TRF does not always represent an individual species or genus.

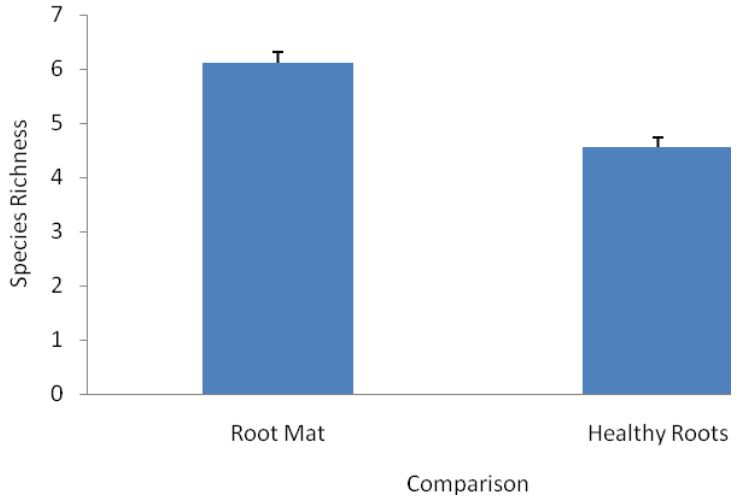


Figure 2.1: Fungal species richness for plants with and without root mat symptoms. Error bars indicate one standard error.

© 2010 Agriculture and Horticulture Development Board

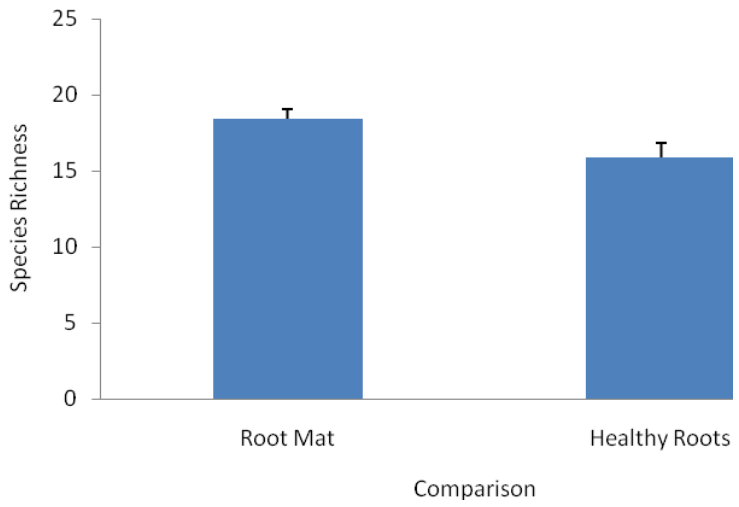


Figure 2.2: Bacterial species richness for plants with and without root mat symptoms. Error bars indicate one standard error.

Agrobacterium biovar 1 strains which are known to cause root mat symptoms in tomato were not identified in the Fragsort output. This could be explained by potential secondary colonizing microbes being a high percentage of the bacterial population so that the *Agrobacterium* TRF is dwarfed and lost as background noise.

Alternatively, it could be due to species of the genus *Agrobacterium* having extremely varied 23S ribosomal regions. *Agrobacterium radiobacter* 23S ribosomal region has not been previously reported; however, a culture of *Agrobacterium radiobacter* known to cause root mat was acquired and sequenced; it was found to give a theoretical fragment peak of 345bp when cut with *Mse*I. But in the case of *Agrobacterium tumefaciens,* which has had several biovar 1 isolates 23S ribosomal regions published, have been shown to give very different TRFs when cut with *Mse*I (319, 381 and 473bp). This could be true for *Agrobacterium radiobacter* and perhaps the reference isolate used the database is not the same as the isolate present in root mat diseased samples and consequently could have a different sized TRF when cut with *Mse*I.

Specific primers could be used on total community DNA to confirm the presence of *Agrobacterium* biovar 1 strains. On the other hand, in order to improve the database, PCRs amplifying the 23S region of root mat samples should be cloned and sequenced then added to the database.

PCA using the covariance matrix was carried out on binary data of comparison samples, but analyzing bacteria and fungi profiles separately.

© 2010 Agriculture and Horticulture Development Board

Factor loading with values > ±0.25 were classed as making a significant contribution to principal components PC1, PC2 and PC3 (Table 4.14 and 4.15 for fungal and bacterial samples respectively). An ANOVA was conducted on PC scores and it was found that PC1 scores were significantly different between root mat roots and healthy roots ($F_{(1, 16)} = 432.88$, P = <0.001) for fungal samples and PC2 was significantly different for bacterial samples ($F_{(1,16)} = 231.02$, P = <0.001). This suggests that the presence or absence of these fragments is significantly important in the grouping of root mat samples and healthy root samples.

It was found that fungal PC1 consisted of 5 significant fragments; 138 (*Plectosphaerella cucumerina*), 180 (*Phytophthora cinnamomi*) and 207 associated with Root Mat samples; 177 and 383 associate with Healthy roots. *Plectosphaerella cucumerina* and *Phytophthora cinnamomi* being identified as significantly contributing to the grouping of root mat samples further back the hypothesis of there being secondary colonization of the diseased roots.

PC2 can be described as 3 fragments; with 352 (*Rhodospirillum spp.*) and 396 *Flavobacterium spp.* being associated with root mat samples; 423 (*Bacillus spp.*) being associated with healthy roots. *Flavobacterium* is the biggest contributor to PC2. Furthermore, certain *Bacillus* spp. are associated with root health, disease suppression and plant growth promotion (Yeoung-Seuk *et al*., 2004; Jian-Hua *et al*., 2004). To confirm the presence of these organisms PCR sample need to be cloned and sequenced.

Table 2.2: Fragments making a significant contribution to PC1, PC2 and PC3, their possible fungal identities and whether they are associated with Root Mat or Healthy samples. (Note: embolden PC loadings are significant between comparisons)

PC Loadings	Fragment Length	Possible Identity	Scores	RM vs. HR
PC1	138	*Plectosphaerella cucumerina*	0.36052	RM
	177	Unidentified	-0.32727	H
	180	*Phytophthora cinnamomi*	0.42428	RM
	207	Unidentified	0.51048	RM
	383	Unidentified	-0.51048	H
PC2	138	*Plectosphaerella cucumerina*	-0.57724	RM
	285	Unidentified	-0.62146	RM
	342	*Gigaspora rosea*	0.41279	H
PC3	177	Unidentified	0.93053	RM
	180	*Phytophthora cinnamomi*	0.28157	RM

© 2010 Agriculture and Horticulture Development Board

Table 2.3: Fragments making a significant contribution to PC1, PC2 and PC3, their possible bacterial identities and whether they are associated with Root Mat or Healthy samples. *(Note: embolden PC loadings are significant between comparisons)*

PC Loadings	Fragment Length	Possible Identity	Scores	RM vs. HR
PC1	98	Unidentified	-0.27765	RM
	160	Unidentified	-0.27765	RM
	168	*Clostridium acetobutylicum*	-0.27765	RM
	195	Unidentified	-0.26835	RM
	340	*Methylobacterium radiotolerans*	0.27765	H
	414	*Geobacter* spp.	0.25	H
PC2	352	*Rhodospirillum* spp.	0.27909	RM
	396	*Flavobacterium* spp.	0.36294	RM
	423	*Bacillus* spp	-0.27397	H
PC3	126	*Pelobacter propionicus*	-0.28615	RM
	157	Unidentified	-0.28615	RM
	158	Unidentified	0.28865	H
	171	*Nitrosomonas* spp.	-0.28615	RM
	231	Unidentified	-0.28615	RM

2.2 Brown roots versus healthy roots

Roots of rockwool grown plants with visibly healthy and visibly discoloured roots were sampled from the same row. As with the previous comparison samples, fungal species richness was higher in plants with diseased roots than in healthy roots ($F_{(1, 16)}$ = 14.88, P = 0.003) (Figure 2.3). This was also true for bacterial species richness ($F_{(1, 16)}$ = 12.79, P = 0.005) (Figure 2.4). This result could also be attributed to the release of utilizable growth substrates from infected tissues encouraging the colonization of opportunistic microbes.

© 2010 Agriculture and Horticulture Development Board

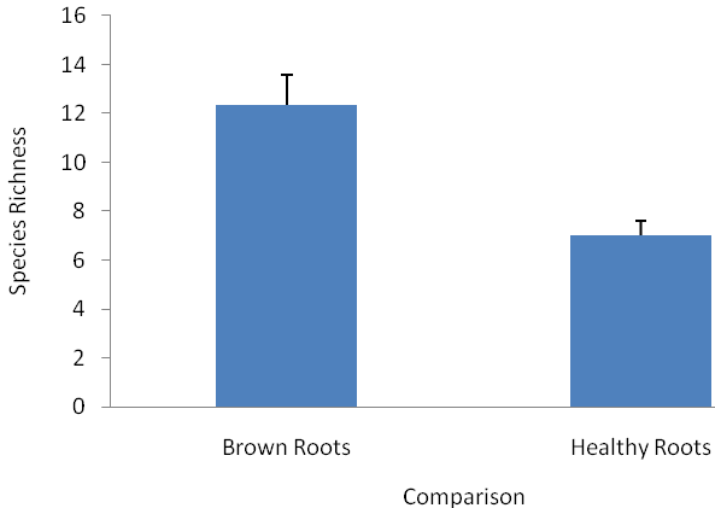


Figure 2.3: Fungal species richness for plants with brown roots and with healthy roots. Error bars indicate one standard error.

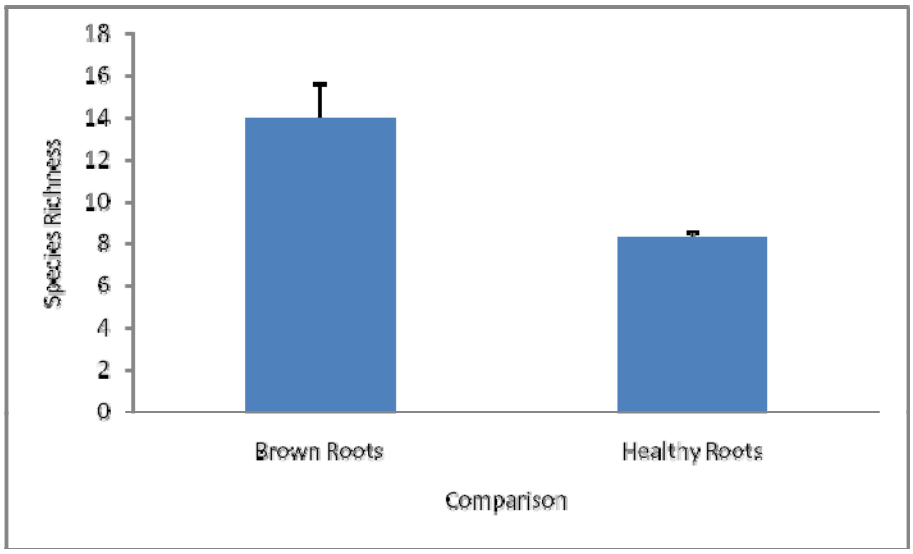


Figure 2.4: Bacterial species richness for plants with brown roots and with healthy roots. Error bars indicate one standard error.

PCA using the covariance matrix was carried out on binary data of comparison samples. Factor loading with values > ±0.25 were classed as making a significant contribution to principle components PC1, PC2 and PC3 (Table 2.4 and 2.5 for fungal and bacterial samples respectively). An ANOVA was conducted on PC scores and it was found that PC1 scores were significantly different between brown roots and healthy roots for fungal samples ($F_{(1, 16)} = 179.01$, $P = <0.001$) and bacterial samples ($F_{(1,16)} = 41.26$, $P = <0.001$). This suggests that the presence or absence of

© 2010 Agriculture and Horticulture Development Board

these fragments is significantly important in the grouping of samples with brown roots and healthy roots.

From the fungal PC1 loadings *Pythium ultimum* was identified as a possible cause for the diseased roots, this finding was backed by the growth of a felty white culture from the plating out of brown roots, with non-septate mycelium and spherical thin walled stalked sporangia.

Table 2.4: Fragments making a significant contribution to PC1, their possible fungal identities and whether they are associated with brown root or healthy root samples.

PC Loadings	Fragment Length	Possible Identity	Scores	BR vs. HR
PC1	285	Unidentified	0.3528	BR
	363	Unidentified	0.26492	BR
	368	*Sporobolomyces* spp.	-0.3528	HR
	389	Unidentified	-0.26046	HR
	428	Unidentified	0.31578	BR
	463	Unidentified	0.30767	BR
	619	*Pythium ultimum*	0.3528	BR
	638	Unidentified	0.3528	BR

Table 2.5: Fragments making a significant contribution to PC1, their possible bacterial identities and whether they are associated with brown root or healthy root samples

PC Loadings	Fragment Length	Possible Identity	Scores	BR vs. HR
PC1	169	*Nitrosomonas* spp	-0.33	HR
	193	*Idiomarina* spp	0.33	BR
	198	*Pseudomonas* spp.	0.33	BR
	352	*Rhodospirillum* spp.	0.26169	BR
	358	*Gloeobacter* spp.	0.32329	BR
	359	Unidentified	0.33	BR
	396	*Flavobacterium*	0.32329	BR
	423	*Frankia* spp.	0.32329	BR
	459	Unidentified	0.32329	BR

2.3 Samples from seed, propagation house and nursery

Samples were taken from seed variety DRC542, plants of variety DRC542 were then sampled at a propagation house just before dispatch and then again at a nursery two weeks after planting. Fungal species richness increased from seed to propagation house and then declined at nursery, however the decline was not significant (P = 0.1190) (Figure 2.5).

© 2010 Agriculture and Horticulture Development Board

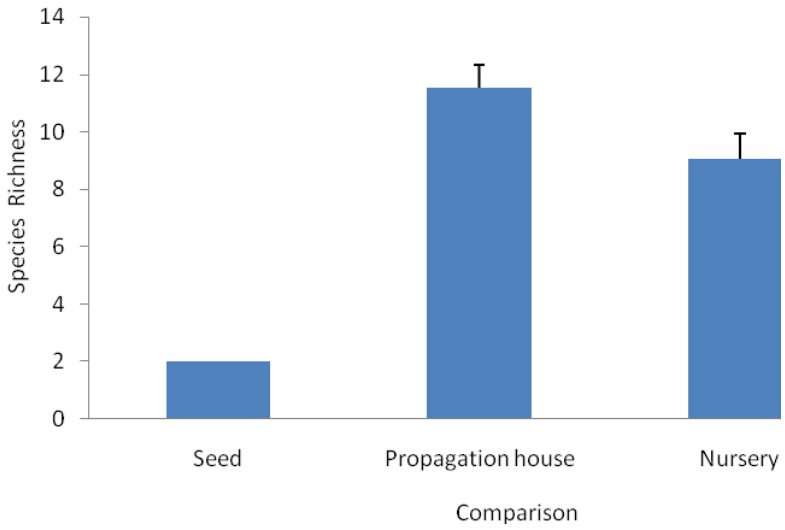


Figure 2.5: Fungal species richness for tomato variety DRC542 sampled at seed, propagation house and nursery. Error bars indicate one standard error.

Comparison = Nursery subtracted from:

Level Comparison	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Propagation	2.487	1.214	2.047	0.1190
seeds	-7.059	1.744	-4.047	0.0010

Comparison = Propagation subtracted from:

Level Comparis	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
seeds	-9.545	1.833	-5.209	0.0001

Bacterial species richness increased from seed to propagation house to nursery. It wasn't significant between seed to propagation house (Figure 2.6).

The fungal fragments making a significant contribution to microbial populations are shown in Table 2.6. These included *Plectrosphaerella cucumerina, Candida albicans* and *Gigaspora rosea* at the propagation stage and a *Fusarium* sp. on the production nursery. However, most of the fragments making a significant contribution were unidentified.

© 2010 Agriculture and Horticulture Development Board

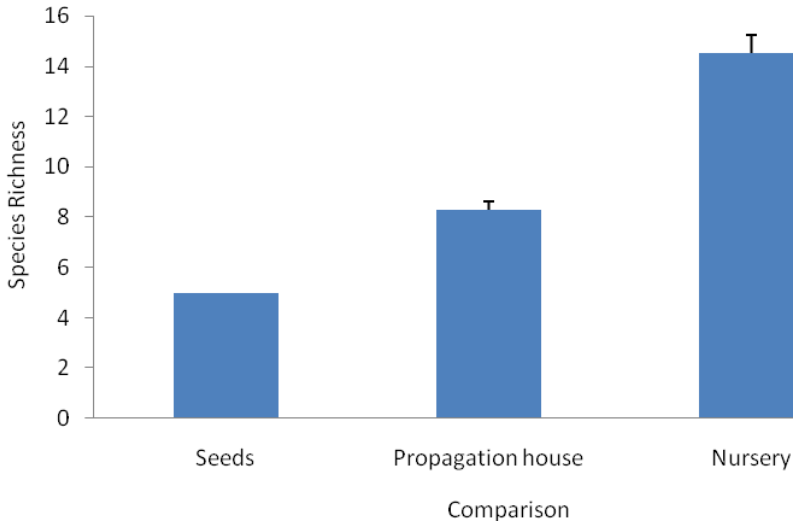


Figure 2.6: Bacterial species richness for tomato variety DRC542 sampled at seed, propagation house and nursery. Error bars indicate one standard error.

Level Comparis	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Propagat	-6.227	0.9340	-6.667	0.0000
seeds	-9.500	1.3490	-7.042	0.0000

Comparis = Propagat subtracted from:

Level Comparis	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
seeds	-3.273	1.425	-2.297	0.0717

© 2010 Agriculture and Horticulture Development Board

Table 2.6: Fragments making a significant contribution to PC1, their possible fungal identities and whether they are associated with seeds, prop house or nursery root samples. (Note: embolden PC loadings are significant between comparisons)

PC Loadings	Fragment Length	Possible Identity	Scores	S vs. PH vs. N
PC1	73	*Fusarium* sp	-0.25	N
	100	Unidentified	0.26057	PH
	267	Unidentified	0.3354	PH
	272	Unidentified	0.28017	PH
	348	*Gigaspora* spp	0.28017	PH
	354	Unidentified	0.26057	PH
	364	Unidentified	0.26057	PH
	367	Unidentified	0.35465	PH
PC2	138	*Plectosphaerella cucumerina*	0.25525	PH+N
	173	Unidentified	0.29124	PH+N
	194	Unidentified	0.25525	PH+N
	206	Unidentified	0.30107	PH+N
	285	Unidentified	0.36873	PH+N
	381	Unidentified	0.40594	PH+N
	434	Unidentified	0.25525	PH+N
PC3	272	Unidentified	-0.32505	PH
	285	Unidentified	0.38652	S + N
	323	*Candida albicans*	-0.40703	PH
	348	*Gigaspora* spp	-0.32505	PH
	364	Unidentified	0.24235	S + N

2.4 Other comparisons

Root samples were also collected in 2009 to examine the effect of Compete Plus monthly drenches, compare new and one year old coir slabs, solution from UV and untreated NFT solution, and roots from yellowing and healthy plants in a crop. These samples have not yet been analysed. Results will be reported in the next annual report.

© 2010 Agriculture and Horticulture Development Board

3. Effect of some biological amendments on microbial populations associated with tomato roots and root disease

Introduction

Various microbial products are marketed for use in commercial crops to increase root health. These include Companion® (*Bacillus subtilis* strain GB03) marketed by Avoncrop Crop Protection and Trianum-P (*Trichoderma harzianum* strain T-22) marketed by Koppert. Addition of earthworms to soil-grown tomato is reported to reduce fusarium wilt disease (Elmer, 2009). Addition of some organic amendments and biological control agents to soil has previously been reported to affect brown and corky root rot of tomato (Giotis *et al,* 2009). The aim of this experiment was to determine the effect of soil treatment with Companion, Trianum-P or worms on fusarium crown and root rot in soil-grown tomato and their effect on microbial populations associated with roots.

Materials and methods

Crop and site details

The experiment was done using a cherry tomato cv. Claree in an unheated glasshouse at ADAS Arthur Rickwood, Cambridgeshire. The variety Claree is not claimed to have resistance to fusarium crown and root rot (*Fusarium oxysporum* f. sp. *radicis-lycopersici*) (FORL). Plants raised in rockwool cubes were transplanted at 4 weeks old into John Innes potting compost no 2 in 3 litre pots, one per pot on 14 April 2009. The plant pots were arranged in trays surrounded by polystyrene packaging material to minimise temperature fluctuation. The pot bases were raised off the concrete floor to prevent movement of drainage water between plants. Plants were trained with one head and fruit was harvested. Plants were fed with potassium nitrate (0.15 kg $KN0_3$/200 L water) at each watering from the start of fruiting. Plants were grown for 14 weeks after potting. A crop diary is given in Appendix 2.

Treatments

1. Unamended soil
2. Unamended soil, inoculated with FORL
3. Earthworms (*Dendrobaena* sp.) added to soil, 12/pot
4. Earthworms added, inoculated with FORL
5. Companion drench at 1.25 ml/L
6. Companion drench, inoculated with FORL
7. Trianum-P drench at 0.05 g/L
8. Trianum-P drench, inoculated with FORL

© 2010 Agriculture and Horticulture Development Board

Worms were incorporated into the soil 7 weeks before planting. They were fed weekly with alfalfa granules at 1-2 g/pot sprinkled over the soil surface. A nylon mesh was tied around pot bases to prevent escape of worms. Aluminium foil was secured around pot tops to reflect light and deter escape of worms. This was removed at 2 weeks after potting to aid watering. Companion and Trianum-P were drenched over the soil surface at 300 ml/pot at 1 and 3 weeks after potting.

Inoculation with FORL

An isolate of FORL was obtained from tomato plants cv. Jack Hawkins in early 2009. A spore suspension in sterile distilled water was prepared from 10 d old cultures on potato dextrose agar and the concentration adjusted to 10^5 spores/ml. Plants were inoculated by pouring 100 ml of this spore suspension over the soil surface at 2 weeks after potting.

Assessments

Plants were examined weekly for wilting or leaf yellowing suggestive of fusarium crown and root rot. Fruit yield from each plot was recorded. Green fruit was included at the final harvest. At the end of the experiment (7 August 2009), plant pots were removed and roots were assessed for extent (0-3 scale) and browning (0-3 scale). The stem base was cut longitudinally on two sides with a scalpel to check for vascular browning suggestive of fusarium crown and root rot.

Root samples for T-RFLP tests

At the end of the experiment, young roots (c. 2 g) were collected from three plants in each plot into small plastic bags. Samples were stored at 4ºC until transfer to Nottingham University for T-RFLP tests.

Experiment design and statistical analysis

The experiment was a randomised block design with fourfold replication. Each plot contained five plants. Results were examined by ANOVA. There was a factorial structure to the treatments, with two factors (inoculation and soil amendment) at two and four levels respectively.

Results and discussion

Fusarium crown and root rot

No symptoms of the disease developed during crop growth and none was evident at the final destructive assessment. Reasons for lack of disease development are unclear. The variety used does not have claimed resistance to FORL; possibly there was insufficient fruit load to stress plants sufficiently. The isolate of FORL was freshly obtained in early 2009 from cv. Jack Hawkins

© 2010 Agriculture and Horticulture Development Board

affected by FCRR symptoms and so should not have lost pathogenicity by sub-culturing. However, possibly it was a weakly pathogenic or non-pathogenic isolate associated with another, more pathogenic isolate on cv. Jack Hawkins; a pathogenicity test was not conducted prior to this experiment.

The experiment was carried out in summer and soil temperatures regularly exceeded 20ºC, occasionally 30ºC for short periods (see Appendix 3). The mean 24h soil temperatures were around 15ºC at the start of the experiment and 20ºC at the end (Appendix 3). The optimum temperature for expression of FCRR is reported to be 15-18 ºC (Jarvis, 1988), so unfavourable temperature is unlikely to account for the lack of disease symptom. Possibly FCRR symptoms may have developed if the crop had been grown longer than 12 weeks, although this seems unlikely given that there was no evidence of infection at this time. Possibly inoculation may have been more successful if plants had been inoculated at an earlier stage. The disease is usually considered to originate from seed-borne infection or infection during propagation. The intention had been to plant and inoculate at the 4-6 leaf seedling stage but the plants supplied were older (6-8 leaf) and in rockwool propagation blocks, and were not inoculated until 2 weeks after potting due to the need first to apply the preventative control treatments.

Plant growth

The plants grew well and were stopped when around 2 m high. Fruit was picked from 26 June 2009 until 21 July 2009. All plants grown in soil amended with worms were initially pale green and thin compared with other treatments. This symptom was suggestive of nitrogen deficiency. Plants grew away from this symptom when feeding with potassium nitrate began on 15 May 2009, although, the stems generally remained thinner. A soil analysis revealed levels of major elements were satisfactory.

Fruit yield over the 4 week fruit picking period was around 800 g/plant. There was no significant difference in yield between treatments (Table 3.1). Mean weight of leaves and stems was around 500 g/plant. The weight of plants grown in soil amended with worms was significantly less than in other treatments, a reflection of the poor growth before plant feeding was started. When data was re-analysed taking account of the factorial structure, inoculation with FORL did not have a significant effect on fruit yield or stem weight (data not shown).

At the end of the experiment, all plants were found to have formed an extensive network of roots throughout the pot of soil. The roots were white in colour and with no evidence of browning or rotting; none of the root ball fell away on de-potting. It was difficult to break the root balls apart in order to examine the larger roots below the stem base. There was no significant difference

© 2010 Agriculture and Horticulture Development Board

between treatments in the extent of white root visible on the outer surface of the root ball (Table 3.2). This remained the case when data was re-examined as a factorial structure; the mean score for extent of white roots on uninoculated plants (2.5) was not significantly greater from that on inoculated plants (2.2) (P = 0.167).

Only four of the 160 plants were found to have vascular browning in the stem base at the end of the experiment, three in inoculated plants and one in an uninoculated plant. The staining was not as dark brown as normally found with FCRR, and no *Fusarium* sp. was recovered from the stained tissues when tested in the laboratory.

Microbial populations associated with roots

T-RFLP analysis was done on a sample of roots from all treatments. Results will be reported in the Final report.

Table 3.1: Effect of biological amendments on growth on tomato cv. Claree in soil – 2009

Treatment		Fruit yield/ plant (g)	Mean stem weight (g)
Amendment	FORL inoculation		
1. Control	-	840	534
2. Control	+	814	516
3. Worms	-	809	467
4. Worms	+	868	483
5. Companion	-	829	534
6. Companion	+	816	523
7. Trianum-P	-	818	532
8. Trianum-P	+	790	504
Significance (20 df)		0.953	0.026
LSD		128.2	43.2

© 2010 Agriculture and Horticulture Development Board

Table 3.2: Effect of biological amendments on occurrence of vascular browning and extent of white root in tomato cv. Claree - 2009

Treatment		Total no plants with stem	Extent of white
Amendment	FORL	base browning (of 20)	roots (0-5)
1. Control	-	0	2.9
2. Control	+	1	2.6
3. Worms	-	1	2.2
4. Worms	+	1	1.8
5. Companion	-	0	2.4
6. Companion	+	0	2.1
7. Trianum-P	-	0	2.8
8. Trianum-P	+	1	2.3
Significance (20 df)			0.380
LSD			0.99

© 2010 Agriculture and Horticulture Development Board

4. 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 (e.g. Gitois *et al*, 2009). 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 were recovered from affected plants 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 in January 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 immediately after planting and again 3 months later.

© 2010 Agriculture and Horticulture Development Board

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 4.1.

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 will be taken per plant to give a density of $4/m^2$.

Table 4.1: 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.
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 a Soil Foodweb Analysis (Laverstoke Park Farm Laboratory Service). Root samples were taken soon after planting, at peak fruit load and in mid-August to determine microbial populations by T-RFLP. Bulk samples of three replicates of each treatment were taken for Soil Foodweb Analysis at first pick and in mid August.

© 2010 Agriculture and Horticulture Development Board

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. At the end of cropping, the number of live heads remaining was assessed. Ten plants in each plot were examined for vascular staining in the stem base. These plants were also forked up, the roots were washed, and the extent and health of roots was estimated. Fruit yield was not recorded.

Results and discussion

Results will be presented in the Final report.

© 2010 Agriculture and Horticulture Development Board

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

Introduction

Rootstocks are used to increase plant vigour and reduce poor growth due to 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 (see section 1). The crop was planted on 29 December 2009

Treatments	Resistances
1. Beaufort (De Ruiter)	HR : ToMV/Fol:0,1/For/Pl/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/Pl/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

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 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 will be taken per plant to give a density of 4/m^2.

© 2010 Agriculture and Horticulture Development Board

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. At the end of cropping, the number of live heads remaining was assessed. Ten plants in each plot were examined for vascular staining in the stem base. These plants were also forked up, the roots were washed, and the extent and health of roots was estimated. Fruit yield was not recorded.

Results and discussion

Results will be presented in the Final report.

TECHNOLOGY TRANSFER

Publications

O'Neill T M (2009). A window on the rhizosphere. *HDC News* **154**, 24-25.

Presentations

Root diseases and their control. WSG Kent, 28 May 2009.

Tomato disease research update. WSG Isle of Wight, 14 July 2009.

Project meetings

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

Project review meeting, Sutton Bonington, 9 October 2009.

CONCLUSIONS

Year 2

1. In 2009 towards the end of cropping, visual examination of plants in 10 UK crops, combined with microscopic examination of root samples, confirmed five root diseases: brown and corky root rot (*Pyrenochaeta lycopersici*), black dot (*Colletotrichum coccodes*), black root rot (*Thielaviopsis basicola*), Fusarium wilt (*Fusarium oxysporum*) and Verticillium wilt (*Verticillium albo-atrum*).

© 2010 Agriculture and Horticulture Development Board

2. Examination of root samples from the 10 crops by a molecular test (T-RFLP) indicated microbial populations contained a total of 92-100 fungal species and over 100 bacterial species.

3. Use of a DNA fragment identification database (Fragsort) identified around 45% of fungal fragments and 72% of bacterial fragments. Twelve of the fungi identified are known pathogens of tomato: *Alternaria solani, Colletotrichum coccodes, Fusarium oxysporum* (or other *Fusarium* species)*, Humicola fuscoatra, Macrophomina phaseolina, Phytophthora* (3 species), *Plectosphaerella cucumerina, Pyrenochaeta lycopersici, Pythium oligandrum* and *Verticillium nigrescens.*

4. T-RFLP indicates the fungi present in greatest abundance on tomato roots were: *Gigaspora rosea* (2.8% of total DNA extracted), *Colletotrichum coccodes* (0.7%), *Macrophomina phaseolina* (0.4%), *Fusarium culmorum*, *Fusarium oxysporum*, *F. oxysporum* f. sp. *lycopersici*, or *F. oxysporum* f. sp. *radicis-lycopersici* (0.25%) and *Cylindrocarpon destructans* (0.24%).

5. *Gigaspora rosea,* an endomycorrhizal fungus that is known to be able to colonise tomato roots, was found on plants in all five growing media.

6. Fragsort produced 347 possible identifications for 90-118 bacterial DNA fragments. Two fragment lengths were identified as possible tomato pathogens, but there were several alternative identifications of non-plant pathogenic bacteria for each fragment.

7. Fungal population diversity on tomato roots, as measured by Simpson's Diversity Index, is significantly influenced by plant age and growing medium. Bacterial population diversity was uninfluenced by these factors, according to Simpson's Diversity Index.

8. Fungal population diversity on young roots from rockwool, coir and NFT crops increased with plant age, while that on soil crops decreased.

9. In an ordination plot of the two principal components of fungal diversity, which accounted for 46% of variation, the samples from rockwool, coir and woodfibre all clustered closely together and distinct both from those in soil and those in NFT.

10. Seven fungal fragments (*Plectosphaerella cucumerina*, *Gigaspora rosae* and four unidentified fungi) accounted for 56% of the variation in fungal diversity between samples.

© 2010 Agriculture and Horticulture Development Board

11. Eight bacterial fragments (*Bacillus* sp., *Rhodobacter sphaeroides, Pasteurella multocida* and five unidentified bacteria) accounted for 48% of the variation in bacterial diversity between samples.

12. T-RFLP tests on root samples indicated the presence of several fungal pathogens which were confirmed by observation on diseased plants including *Colletotrichum coccodes, Fusarium oxysporum* and *Pyrenochaeta lycopersici.*

13. T-RFLP tests on root samples did not detect several fungi which were observed to have caused disease in the monitored plants, including *Thielaviopsis basicola* and *Verticillium albo-atrum*.

14. T-RFLP tests on root samples analysed by the Fragsort database indicated the presence of some fungal pathogens which are 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.

15. In the 10 crops examined in 2009, T-RFLP tests indicated that five fungal pathogens were present in more than one crop. These were: *Plectosphaerella cucumerina* (7 crops), *Colletotrichum coccodes* (5 crops), *Fusarium oxysporum* (3 crops), *Pyrenochaeta lycopersici* (2 crops) and *Alternaria solani* (2 crops). The importance of *P. cucumerina* as a pathogen in UK crops warrants further examination.

16. 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 whether T-RFLP can be used to predict root health.

17. Both fungal and bacterial species richness was greater in plants with root mat symptoms than without, possibly a reflection of secondary colonisation of damaged tissues.

18. Fungal and bacterial species richness was greater in plants with brown roots that in white roots from adjacent plants. *Pythium ultimum* was confirmed at a high level in the brown roots by T-RFLP.

© 2010 Agriculture and Horticulture Development Board

REFERENCES

Anthony R M, Brown T J & French G L (2000). Rapid diagnosis of bacteria by universal amplification of 23S ribosomal DNA followed by hybridization to an oligonucleotide array. *Journal of Clinical Microbiology* **38**, 781-788.

Buir T J & Caesar A (1984). Beneficial plant bacteria. *Critical Review in Plant Science* **2**, 1–20.

Chao A & Shen T J (2003). Nonparametric estimation of Shannon's index of diversity when there are unseen species in sample. *Environmental and Ecological Statistics* **10**, 429–443.

Cullen D W & Hirsch P R (1998). Simple and rapid method for direct extraction of microbial DNA from soil for PCR. *Soil Biology and Biochemistry* **30**, 983-993.

Culman S W, Gauch H G, Blackwood C B & Thies J E (2008). Analysis of T-RFLP data using analysis of variance and ordination methods: A comparative study. *Journal of Microbiological Methods* **75**, 55-63

Dunbar J, Ticknor LO and Kuske CR (2001). Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. *Applied Environmental Microbiology* **67**, 190–197.

Elmer W H (2009). Influence of earthworm activity on soil microbes and soilborne diseases of vegetables. *Plant Disease* **93**, 175-179.

Filion M, Hamelin RC, Bernier L and St-Arnaud M (2004). Molecular profiling of rhizosphere microbial communities associated with healthy and diseased Black Spruce (*Picea mariana*) seedlings grown in a nursery. *Applied and Environmental Microbiology* **70**, 3541–3551.

Gardener BBMS and Weller DM (2001). Changes in populations of rhizosphere bacteria associated with take-all disease of wheat. *Applied and Environmental Microbiology* **67**, 4414-4425.

Giotis C, Markelou E, Theodoropoulou A, Toufexi, E, Hudson R, Shotton P, Shiel R, Cooper J & Leifert C (2009). Effect of soil amendments and biological control agents (BCAs) on soil-borne root diseases caused by *Pyrenochaeta lycopersici* and *Verticillium albo-atrum* in organic greenhouse tomato production systems. *European Journal of Plant Pathology* **123**, 387-400.

© 2010 Agriculture and Horticulture Development Board

Griffiths B S, Ritz K, Wheatley R, Kuan H L, Boag B, Christensen S, Ekelund F, Sorensen S J, Muller S & Bloem J (2001). An examination of the biodiversity-ecosystem function relationship in arable soil microbial communities. *Soil Biology and Biochemistry* 33, 1713-1722.

Head I M, Saunders J R & Pickup R W (1998). Microbial evolution, diversity, and ecology: A decade of ribosomal RNA analysis of uncultivated microorganisms. *Microbial Ecology* **35**, 1-21.

Heywood V H (1995). Global biodiversity assessment. *Cambridge University Press*, Cambridge.

Jarvis WR (1988). Fusarium crown and root rot of tomato. *Phytoprotection* 69, 49-64.

Jian-Hua G, Hong-Ying Qi, Ya-Hui G, Hong-Lian G, Long-Ying G, Li-Xin Z and Ping-Hua Su (2004). Biocontrol of tomato wilt by plant growth-promoting rhizobacteria. *Biocontrol* **29**, 66-72.

Magurran A E (2004). Measuring Biological Diversity. *Blackwell Publishing*, Oxford.

Michel F C & Sciarini S (2003). T-RFLP FRAGSORT: a computer program to correlate multiple 16S rRNA gene T-RFLP profiles with corresponding in silico amplification and digestions of ribosomal database project II alignments, abstract. N-289. 103rd Meeting American Society for Microbiology, Washington, DC.

Pascoe I G, Nancarrow R J & Copes C J (1984). *Fusarium tabacinum* on tomato and other hosts in Australia. *Transactions British Mycological Society*, **82**, 343-345.

Paulitz T C (1997). Biological control of root pathogens in soilless and hydroponic systems. *Horticultural Science* **32**, 193–196.

Pio C A, Castro L M, Cerqueira M A, Santos I M, Belchoir F & Salgueiro M L (1996). Source assessment of particulate air pollutants measured at the Southwest European coast. *Atmospheric Environment* **30**, 3309-3320.

Ramette A (2007). Multivariate analyses in microbial ecology. *FEMS Microbiology Ecology* **62**, 142-160.

Scervino J M, Ponce M A, Erra-Bassells R, Vierheilig H, Ocampo J A & Godeas A (2005). Arbuscular mycorrhizal colonization of tomato by *Gigaspora* and *Glomus* species in the presence of root flavonoids. *Journal of Plant Physiology* **162**, 625-633.

© 2010 Agriculture and Horticulture Development Board

Simpson E H (1949). Measurement of diversity. *Nature* **163**, 688.

Sharma S (1995). Applied multivariate techniques. Wiley Publishing LTD, New York, 58–87.

Sharma A, Wray V & Johri B (2007). Rhizosphere *Pseudomonas* sp strains reduce occurrence of pre- and post-emergence damping-off in Chile and tomato in Central Himalayan region. *Archives of Microbiology* **187**, 321-335.

Swinnen J, Van Veen J A & Merck R (1994). 14–C pulse labelling of field-grown spring wheat: an evaluation of its use in rhizosphere carbon budget estimations. *Soil Biology and Biochemistry* **26**, 161–170.

Uecker F A (1993). Development and cytology of *Plectosphaerella cucumerina. Mycologia* **3**, 470-470.

Yeoung-Seuk B, Kyungseok P and Choong-Hoe K (2004). *Bacillus* spp. as Biocontrol Agents of Root Rot and Phytophthora Blight on Ginseng. *Journal of Plant Pathology* **20**, 63-66

© 2010 Agriculture and Horticulture Development Board

Appendix 1. Bacteria identified on routine samples - 2009

Bacterial organisms identified in Fragsort from routine samples collected in 2009, showing TRF size when cut with *Mse*I and *Hae*III and relative abundance (%).

Organism Name	*Hae*III TRF size	*Mse*I TRF size	TRF %
Streptomyces avermitilis MA-4680, Streptomyces coelicolor A3(2) or *Streptomyces griseus subsp. griseus NBRC 13350*	95	458	0.08
Mycobacterium ulcerans Agy99	96	193	0.12
Renibacterium salmoninarum ATCC 33209	96	455	0.12
Leifsonia xyli subsp. xyli str. CTCB07	96	457	0.12
Kocuria rhizophila DC2201	96	458	0.08
Clavibacter michiganensis subsp. michiganensis NCPPB 382 or *Nocardia farcinica IFM 10152*	96	459	0.08
Mycobacterium bovis AF2122/97, Mycobacterium gilvum PYR-GCK, Mycobacterium sp. MCS, Mycobacterium tuberculosis CDC1551 or *Rhodococcus sp. RHA1*	96	460	0.08
Arthrobacter sp. FB24, Mycobacterium avium 104 or *Mycobacterium vanbaalenii PYR-1*	96	461	0.01
Arthrobacter aurescens TC1 or *Mycobacterium smegmatis*	96	462	0.01
Bifidobacterium adolescentis ATCC 15703	97	435	0.01
Tropheryma whipplei TW08/27	97	459	0.08
Alkaliphilus oremlandii OhILAs	118	374	0.05
Clostridium thermocellum ATCC 27405	119	415	0.28
Leptospira borgpetersenii	119	436	0.01
Leptospira interrogans serovar Lai str. 56601	119	437	0.01
Thermotoga maritima MSB8, Thermotoga petrophila RKU-1 or *Thermotoga sp. RQ2*	119	447	0.01
Prochlorococcus marinus str. MIT 9211	123	393	0.07
Pelobacter propionicus DSM 2379	125	125	0.04
Xanthomonas axonopodis pv. citri str. 306, Xanthomonas campestris pv. campestris str. 8004 or *Xanthomonas oryzae pv. oryzae KACC10331*	125	133	0.04
Stenotrophomonas maltophilia R551-3 or *Xylella fastidiosa 9a5c*	125	199	0.11
Haemophilus ducreyi 35000HP	127	374	0.21
Kineococcus radiotolerans SRS30216	129	461	0.01
Moorella thermoacetica ATCC 39073	133	450	0.03
Methylacidiphilum infernorum V4	148	139	0.02

© 2010 Agriculture and Horticulture Development Board

Organism Name	*Hae*III TRF size	*Mse*I TRF size	TRF %
Bifidobacterium longum DJO10A	150	437	0.01
Caulobacter sp. K31	154	291	0.33
Phenylobacterium zucineum HLK1	154	410	0.22
Dinoroseobacter shibae DFL 12 or *Silicibacter pomeroyi DSS-3*	155	110	0.16
Magnetospirillum magneticum AMB-1	155	134	0.03
Rhodospirillum centenum SW	155	354	0.05
Maricaulis maris MCS10	155	365	1
Sinorhizobium meliloti 1021	155	472	0.01
Rhizobium etli CFN 42 or *Rhizobium leguminosarum bv. trifolii WSM2304*	156	183	0.33
Nitrobacter hamburgensis X14 or *Rhodopseudomonas palustris BisA53*	156	294	0.05
Xanthobacter autotrophicus Py2	156	296	0.3
Sphingomonas wittichii RW1	156	335	0.58
Gluconacetobacter diazotrophicus PAl 5 or *Parvibaculum lavamentivorans DS-1*	156	356	1.11
Rhodospirillum rubrum ATCC 11170	156	357	1.89
Beijerinckia indica subsp. indica ATCC 9039	156	386	0.03
Nitrobacter winogradskyi Nb-255	156	423	0.04
Mesorhizobium loti MAFF303099	156	465	0.02
Bradyrhizobium sp. BTAi1	156	466	0.01
Bradyrhizobium japonicum USDA 110	156	477	0.02
Psychrobacter sp. PRwf-1	157	110	0.16
Alcanivorax borkumensis SK2, *Chromohalobacter salexigens DSM 3043* or *Hahella chejuensis KCTC 2396*	157	198	0.93
Novosphingobium aromaticivorans DSM 12444	157	402	0.04
Halorhodospira halophila SL1, *Pseudomonas aeruginosa PA7*, *Pseudomonas entomophila L48*, *Pseudomonas fluorescens Pf-5*, *Pseudomonas stutzeri A1501* or *Pseudomonas syringae pv. phaseolicola 1448A*	158	199	0.59
Paracoccus denitrificans PD1222	159	110	0.16
Rhodobacter sphaeroides 2.4.1	159	312	3.23
Methylobacterium radiotolerans JCM 2831	159	338	3.9
Methylobacterium populi BJ001	159	410	0.22
Methylobacterium extorquens PA1	159	418	0.01
Aeromonas salmonicida subsp. salmonicida A449	162	200	1.85
Chloroherpeton thalassium ATCC 35110	163	391	0.03
Chlorobaculum parvum NCIB 8327	163	392	0.02
Chlorobium tepidum TLS	163	396	0.2
Desulfovibrio desulfuricans	163	414	0.68
Symbiobacterium thermophilum IAM 14863	163	417	0.09

© 2010 Agriculture and Horticulture Development Board

Organism Name	*Hae*III TRF size	*Mse*I TRF size	TRF %
Streptococcus suis 05ZYH33	164	139	0.02
Streptococcus thermophilus CNRZ1066	164	198	0.05
Rhodopirellula baltica SH 1	164	383	0.05
Geobacter bemidjiensis Bem, *Geobacter lovleyi SZ*, *Geobacter metallireducens GS-15* or *Geobacter sulfurreducens PCA*	164	414	0.05
Bacillus clausii KSM-K16, *Exiguobacterium sibiricum 255-15* or *Geobacillus thermodenitrificans NG80-2*	164	419	0.05
Geobacillus kaustophilus HTA426	164	421	0.05
Sorangium cellulosum 'So ce 56'	164	452	0.05
Photorhabdus luminescens subsp. laumondii TTO1	165	308	0.11
Enterobacter sakazakii ATCC BAA-894	165	371	0.11
Desulfotalea psychrophila LSv54	165	379	0.02
Gramella forsetii KT0803	165	395	0.11
Rubrobacter xylanophilus DSM 9941	165	459	0.08
Geobacter uraniireducens Rf4	166	186	0.11
Dechloromonas aromatica RCB, *Delftia acidovorans SPH-1* or *Rhodoferax ferrireducens T118*	166	191	0.07
Thiobacillus denitrificans ATCC 25259	166	333	0.02
Polaromonas naphthalenivorans CJ2	166	366	0.11
Leptothrix cholodnii SP-6 or *Methylibium petroleiphilum PM1*	166	367	0.11
Methylobacillus flagellatus KT or *Polaromonas sp. JS666*	166	368	0.11
Verminephrobacter eiseniae EF01-2	166	369	0.11
Bordetella avium 197N, *Bordetella bronchiseptica RB50*, *Bordetella parapertussis 12822*, *Bordetella pertussis Tohama I*, *Bordetella petrii DSM 12804*, *Burkholderia pseudomallei 1106a*, *Burkholderia thailandensis E264*, *Cupriavidus taiwanensis* or *Nitrosospira multiformis ATCC 25196*	166	370	0.11

© 2010 Agriculture and Horticulture Development Board

Organism Name	*Hae*III TRF size	*Mse*I TRF size	TRF %
Burkolderia cepacia, *Burkholderia cenocepacia AU 1054,* *Burkholderia multivorans ATCC 17616,* *Burkholderia phymatum STM815,* *Burkholderia sp. 383,* *Burkholderia vietnamiensis G4,* *Ralstonia eutropha H16,* *Ralstonia metallidurans CH34,* *Ralstonia pickettii 12J* or *Ralstonia solanacearum GMI1000*	166	371	0.11
Herpetosiphon aurantiacus ATCC 23779	167	191	0.07
Natranaerobius thermophilus JW/NM-WN-LF	167	398	2.01
Pelotomaculum thermopropionicum SI	167	421	1.28
Saccharopolyspora erythraea NRRL 2338	167	454	0.02
Desulfitobacterium hafniense Y51	168	326	0.04
Heliobacterium modesticaldum Ice1	168	408	0.23
Listeria innocua Clip11262, *Listeria monocytogenes EGD-e* or *Listeria welshimeri serovar 6b str. SLCC5334*	168	418	0.01
Bacillus amyloliquefaciens FZB42, *Bacillus licheniformis ATCC 14580,* *Bacillus pumilus SAFR-032* or *Bacillus subtilis subsp. subtilis str. 168*	168	419	0.07
Frankia alni ACN14a or *Frankia sp. CcI3*	168	425	0.1
Corynebacterium diphtheriae NCTC 13129	170	333	0.02
Dictyoglomus thermophilum H-6-12	170	449	0.01
Caldicellulosiruptor saccharolyticus DSM 8903	171	368	0.41
Mycobacterium abscessus	171	462	0.01
Thermosipho melanesiensis BI429	172	414	0.83
Corynebacterium urealyticum DSM 7109	173	446	0.01
Hyphomonas neptunium ATCC 15444	192	288	0.33
Neorickettsia sennetsu str. Miyayama	193	358	0.6
Candidatus Ruthia magnifica	194	125	0.04
Treponema pallidum subsp. pallidum SS14	196	426	0.1
Caulobacter crescentus CB15	198	291	0.07
Granulibacter bethesdensis CGDNIH1	199	118	0.02
Brucella canis ATCC 23365	199	341	0.06
Sinorhizobium medicae WSM419	199	343	0.06

© 2010 Agriculture and Horticulture Development Board

Organism Name	*Hae*III TRF size	*Mse*I TRF size	TRF %
Rickettsia akari str. Hartford, *Rickettsia canadensis str. McKiel,* *Rickettsia conorii str. Malish 7,* *Rickettsia felis URRWXCal2,* *Rickettsia massiliae MTU5,* *Rickettsia rickettsii str. 'Sheila Smith'* or *Rickettsia typhi str. Wilmington*	199	358	0.6
Rickettsia bellii OSU 85-389	199	359	0.97
Brucella abortus S19, *Brucella abortus bv. 1 str. 9-941,* *Brucella ovis ATCC 25840* or *Brucella suis 1330*	199	518	0.01
Brucella melitensis biovar Abortus 2308	199	519	0.01
Orientia tsutsugamushi str. Boryong	200	119	0.02
Zymomonas mobilis subsp. mobilis ZM4	200	130	0.15
Candidatus Pelagibacter ubique HTCC1062	200	296	0.3
Sphingopyxis alaskensis RB2256	200	335	0.58
Gluconobacter oxydans 621H	200	354	0.05
Methylobacterium sp. 4-46	200	411	0.04
Bartonella bacilliformis KC583, *Bartonella henselae str. Houston-1,* *Bartonella quintana str. Toulouse* or *Bartonella tribocorum CIP 105476*	201	118	0.02
Oligotropha carboxidovorans OM5	201	423	0.04
Propionibacterium acnes KPA171202	210	392	0.02
Thermus thermophilus HB27	212	400	0.11
Pelobacter carbinolicus DSM 2380	212	413	0.11
Deinococcus geothermalis DSM 11300	227	390	0.03
Anaplasma marginale str. St. Maries	322	359	0.03
Cytophaga hutchinsonii ATCC 33406	328	202	0.03
Anaplasma phagocytophilum HZ	334	359	0.03
Shewanella denitrificans OS217, *Shewanella oneidensis MR-1,* *Shewanella putrefaciens CN-32,* *Shewanella sp. ANA-3* or *Shewanella sp. MR-4*	335	199	0.03
Thiomicrospira crunogena XCL-2	342	121	0.03
Herminiimonas arsenicoxydans, *Janthinobacterium sp. Marseille* or *Nitratiruptor sp. SB155-2*	342	367	0.03
Azoarcus sp. BH72, *Burkholderia phytofirmans PsJN* or *Burkholderia xenovorans LB400*	345	370	1.51
Trichodesmium erythraeum IMS101	347	395	0.2

© 2010 Agriculture and Horticulture Development Board

Organism Name	*Hae*III TRF size	*Mse*I TRF size	TRF %
Psychrobacter arcticus 273-4 or *Psychrobacter cryohalolentis K5*	349	110	0.16
Wolbachia endosymbiont	355	118	0.02
Saccharophagus degradans 2-40	356	110	0.16
Alteromonas macleodii 'Deep ecotype'	358	199	0.12
Pseudoalteromonas atlantica T6c	359	120	0.2
Pseudoalteromonas haloplanktis TAC125	360	120	0.2
Vibrio harveyi ATCC BAA-1116	360	121	0.2
Aliivibrio salmonicida LFI1238 or *Vibrio fischeri ES114*	360	191	0.07
Shewanella amazonensis SB2B, *Shewanella halifaxensis HAW-EB4*, *Shewanella loihica PV-4*, *Shewanella pealeana ATCC 700345*, *Shewanella sediminis HAW-EB3*, *Shewanella woodyi ATCC 51908*, *Vibrio cholerae O1 biovar El Tor str. N16961*, *Vibrio parahaemolyticus RIMD 2210633* or *Vibrio vulnificus CMCP6*	360	199	0.35
Synechococcus sp. CC9605	360	368	0.35
Synechococcus elongatus PCC 6301 or *Synechococcus sp. CC9311*	360	392	0.02
Synechococcus sp. CC9902	360	393	0.21
Marinomonas sp. MWYL1	361	122	0.05
Idiomarina loihiensis L2TR	361	193	0.19
Marinobacter aquaeolei VT8	361	198	0.19
Photobacterium profundum SS9	361	199	0.19
Prochlorococcus marinus str. AS9601	361	394	0.19
Colwellia psychrerythraea 34H	362	121	0.2
Cellvibrio japonicus Ueda107 or *Methylococcus capsulatus str. Bath*	362	199	0.59
Sulfurimonas denitrificans DSM 1251	362	362	0.6
Acinetobacter baumannii ACICU, *Acinetobacter baumannii ATCC 17978*, *Acinetobacter baumannii AYE*, *Acinetobacter baumannii SDF*, *Aeromonas hydrophila* or *Pseudomonas mendocina ymp*	363	199	0.59
Candidatus Phytoplasma australiense	365	200	1.66
Nitrosomonas europaea ATCC 19718 or *Nitrosomonas eutropha C91*	368	170	0.04
Chromobacterium violaceum ATCC 12472	368	368	0.1
Francisella philomiragia or *Francisella tularensis subsp. holarctica*	369	369	0.1
Buchnera aphidicola	370	308	0.3

© 2010 Agriculture and Horticulture Development Board

Organism Name	*Hae*III TRF size	*Mse*I TRF size	TRF %
Serratia proteamaculans 568	371	125	0.04
Polynucleobacter necessarius STIR1 or *Polynucleobacter sp. QLW-P1DMWA-1*	371	191	0.07
Baumannia cicadellinicola, *Enterobacter sp. 638*, *Escherichia coli 536*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Salmonella typhimurium LT2*, *Shigella boydii CDC 3083-94*, *Shigella dysenteriae Sd197* or *Shigella flexneri 2a str. 2457T*	371	308	0.3
Citrobacter koseri ATCC BAA-895, *Erwinia tasmaniensis*, *Sodalis glossinidius str. 'morsitans'*, *Yersinia pestis Angola* or *Yersinia pseudotuberculosis IP 31758*	371	371	2.46
Mannheimia succiniciproducens MBEL55E, *Neisseria gonorrhoeae FA 1090*, *Neisseria meningitidis 053442* or *Pasteurella multocida subsp. multocida str. Pm70*	373	373	6.45
Haemophilus influenzae	374	374	0.21
Elusimicrobium minutum Pei191	378	121	0.04
Campylobacter fetus subsp. fetus 82-40	388	198	0.02
Helicobacter hepaticus ATCC 51449	388	340	0.02
Thermosynechococcus elongatus BP-1	389	129	0.02
Arcobacter butzleri RM4018	390	139	0.02
Gloeobacter violaceus PCC 7421	390	359	0.02
Campylobacter hominis ATCC BAA-381 or *Campylobacter jejuni RM1221*	390	390	0.02
Campylobacter concisus 13826 or *Pelodictyon luteolum DSM 273*	391	391	0.03
Jannaschia sp. CCS1	392	291	0.05
Anabaena variabilis ATCC 29413 or *Pelodictyon phaeoclathratiforme BU-1*	392	392	0.02
Microcystis aeruginosa NIES-843	393	121	0.06
Nostoc sp. PCC 7120 or *Synechocystis sp. PCC 6803*	393	393	0.06
Candidatus Sulcia muelleri GWSS	394	135	0.03
Chlorobium chlorochromatii CaD3	394	394	0.03
Chlorobium limicola DSM 245	395	395	0.2
Roseobacter denitrificans OCh 114	396	110	0.16

© 2010 Agriculture and Horticulture Development Board

Organism Name	*Hae*III TRF size	*Mse*I TRF size	TRF %
Chlorobium phaeobacteroides BS1, Flavobacterium johnsoniae UW101, Flavobacterium psychrophilum JIP02/86 or *Prosthecochloris aestuarii DSM 271*	396	396	0.2
Silicibacter sp. TM1040	397	110	0.16
Bacteroides thetaiotaomicron VPI-5482	400	127	0.02
Bacteroides vulgatus ATCC 8482	401	401	0.02
Fervidobacterium nodosum Rt17-B1	402	402	0.02
Candidatus Amoebophilus asiaticus 5a2	403	127	0.02
Clostridium phytofermentans ISDg	403	403	0.02
Streptococcus agalactiae 2603V/R	405	127	0.02
Streptococcus pyogenes M1 GAS	405	139	0.02
Clostridium acetobutylicum ATCC 824	406	168	0.04
Clostridium novyi NT	406	406	0.02
Clostridium botulinum A str. ATCC 19397	407	123	0.09
Chlamydophila abortus S26/3, Chlamydophila caviae GPIC, Chlamydophila felis Fe/C-56 or *Chlamydophila pneumoniae AR39*	407	197	0.09
Clostridium perfringens ATCC 13124	407	344	0.06
Clostridium difficile 630	408	136	0.07
Chlamydia muridarum Nigg	408	408	0.09
Chlamydia trachomatis 434/Bu	410	410	0.09
Enterococcus faecalis V583 or *Thermodesulfovibrio yellowstonii DSM 11347*	411	411	0.04
Mycoplasma agalactiae PG2	412	130	0.09
Desulfotomaculum reducens MI-1	412	412	0.09
Mycoplasma pulmonis UAB CTIP	414	383	0.09
Desulfovibrio vulgaris DP4 or *Desulfovibrio vulgaris str. Hildenborough*	414	414	0.31
Mycoplasma mobile 163K	416	290	0.31
Mycoplasma arthritidis 158L3-1	416	378	0.02
Lactobacillus salivarius UCC118 or *Myxococcus xanthus DK 1622*	416	416	0.09
Lactobacillus fermentum IFO 3956 or *Lactobacillus reuteri DSM 20016*	417	417	0.09
Staphylococcus aureus RF122, Staphylococcus epidermidis ATCC 12228, Staphylococcus haemolyticus JCSC1435 or *Staphylococcus saprophyticus*	418	418	0.01
Lysinibacillus sphaericus C3-41	419	388	0.03

© 2010 Agriculture and Horticulture Development Board

Organism Name	*Hae*III TRF size	*Mse*I TRF size	TRF %
Bacillus anthracis str. 'Ames Ancestor', *Bacillus anthracis str. Ames,* *Bacillus cereus ATCC 10987,* *Bacillus halodurans C-125,* *Bacillus thuringiensis serovar konkukian str. 97-27* or *Bacillus weihenstephanensis KBAB4*	419	419	0.03
Roseiflexus castenholzii DSM 13941 or *Roseiflexus sp. RS-1*	427	427	0.01
Anaeromyxobacter dehalogenans 2CP-C	429	429	0.01
Hydrogenobaculum sp. Y04AAS1	438	438	0.01
Leptospira biflexa	441	119	0.02
Borrelia afzelii Pko, *Borrelia burgdorferi B31* or *Borrelia garinii PBi*	444	120	0.03
Dehalococcoides sp. BAV1	446	109	0.02
Borrelia duttonii Ly, *Borrelia hermsii DAH,* *Borrelia recurrentis A1* or *Borrelia turicatae 91E135*	446	121	0.03
Dehalococcoides ethenogenes 195	446	137	0.03
Sulfurihydrogenibium sp. YO3AOP1	447	417	0.03
Syntrophomonas wolfei	455	455	0.03
Salinispora arenicola CNS-205	457	457	0.03
Frankia sp. EAN1pec	458	425	0.08
Corynebacterium efficiens YS-314, *Salinispora tropica CNB-440* or *Syntrophus aciditrophicus SB*	458	458	0.08
Corynebacterium glutamicum ATCC 13032	460	460	0.08

© 2010 Agriculture and Horticulture Development Board

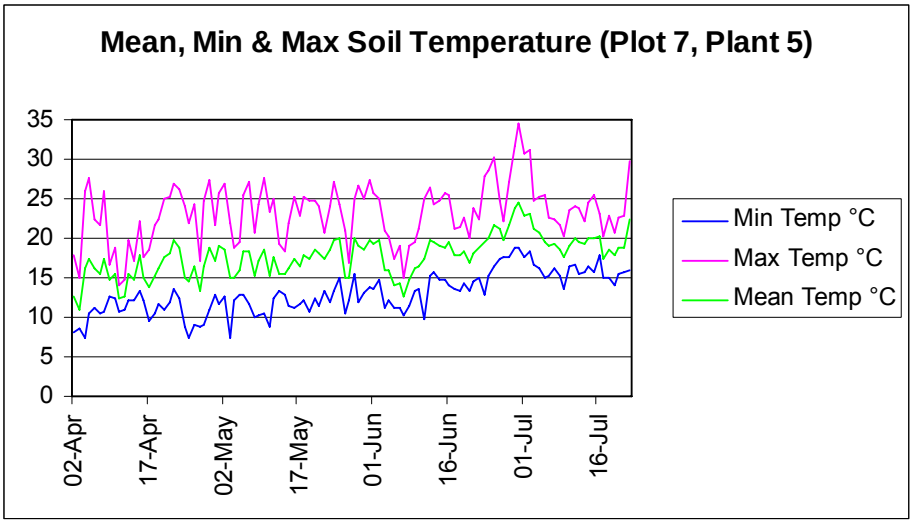
Appendix 2. Trial diary, ADAS 2009

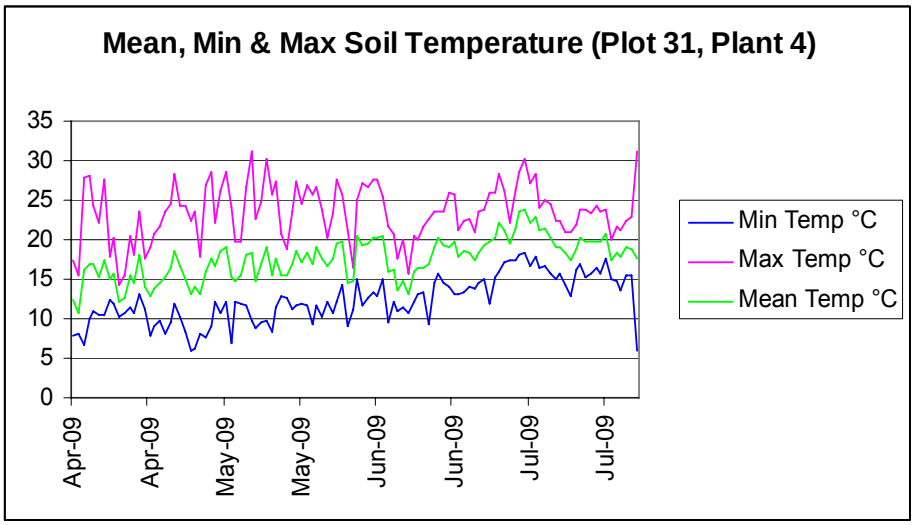
Trial diaries for tomato: the effect of biological amendments on microbial populations associated with roots and the occurrence of root disease – ADAS Arthur Rickwood

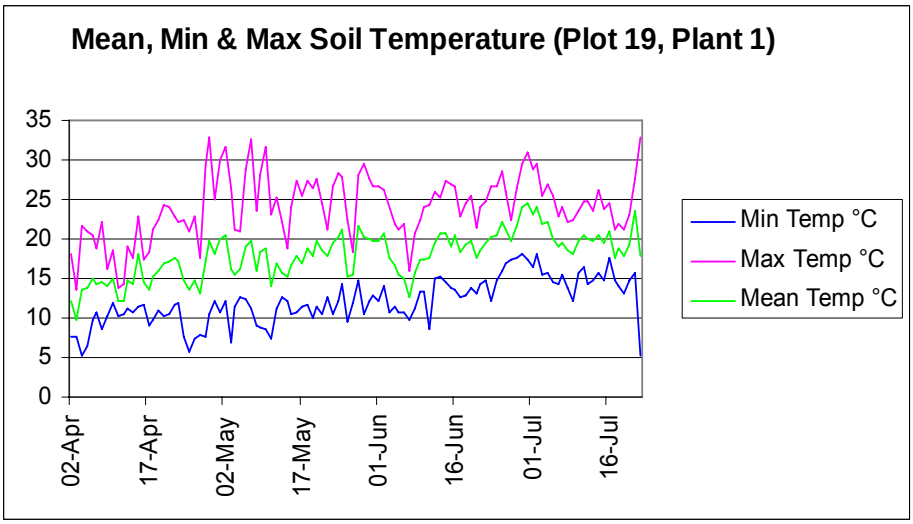
Date	Action
20/02/09	Trial set up as per protocol. 12 Dendrobaena worms added to 40 x 3 L pots containing John Innes No.2 soil.
14/04/09	180 tomato plugs in rockwool cv. Claree potted up into 3 L pots containing John Innes No.2 (including worm pots from 20/02/09).
24/04/09	Plots requiring Treatments 5 & 6 drenched with Companion (rate 1.25 ml/L). Plots requiring Treatments 7 & 8 drenched with Trianum-P (rate 0.05 g/L). Plots requiring Treatments 1 – 4 were watered.
01/05/09	Inoculum prepared from plates of *Fusarium oxysporum f.sp. radicis-lycopersici* to give a suspension of $1x10^5$. Plants requiring T2, T4, T6 & T8 inoculated.
08/05/09	Plots requiring Treatments 5 & 6 drenched with Companion (rate 1.25 ml/L). Plots requiring Treatments 7 & 8 drenched with Trianum-P (rate 0.05 g/L). Plots requiring Treatments 1 – 4 were watered.
15/05/09	First fruit - Plants to be liquid fed with potassium nitrate only (150 g potassium nitrate per 200 L water).
22/06/09	Plants 'stopped'.
26/06/09	1st fruit harvest.
30/06/09	2nd fruit harvest.
03/07/09	3rd fruit harvest.
07/07/09	4th fruit harvest.
10/07/09	5th fruit harvest.
14/07/09	6th fruit harvest.
17/07/09	7th fruit harvest.
21/07/09	8th fruit harvest - final (ripe and green fruit). Destructive assessment of plant tops – weight and vascular staining.
23/07/09	Destructive assessment of plant roots – browning of root ball and inner roots. Worms present / absent T3 + T4.
24/07/09	Plating out of destructive assessment samples.
28/07/09	Stem and root samples sent to Sarah Deery (Nottingham University) for analysis by T-RFLP.
07/08/09	Data sent to Chris Dyer for analysis.

© 2010 Agriculture and Horticulture Development Board

Appendix 3. Soil temperatures – ADAS Arthur Rickwood trial







© 2010 Agriculture and Horticulture Development Board