Horticultural Development Company

Grower summary

PC 281

Protected tomato: monitoring rhizosphere micro-organisms to improve understanding and management of root diseases

Annual Report 2010

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Further information

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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 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).

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

		Growing medium and crop reference number									
Fragment length (bp)	Probable identity [a]	RW		Soil		NFT		Coir		WF	
		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.

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).

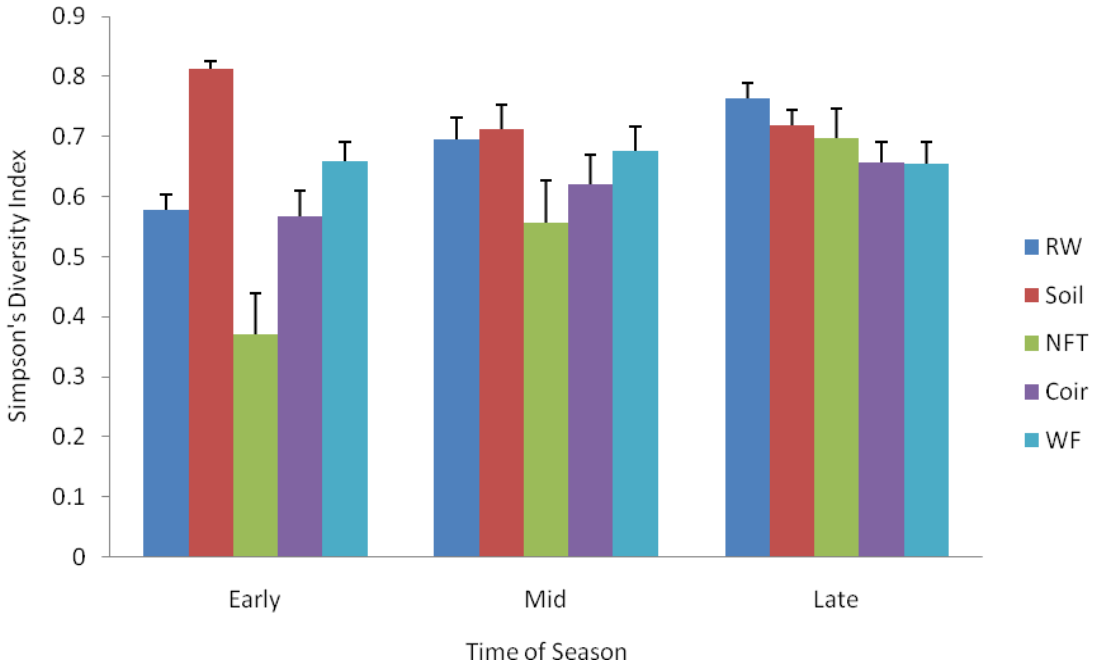

Figure 1: Effect of sample time (plant age) and growth medium on fungal diversity calculated using the Simpson's Diversity equation for all 10 crops at 3 sampling times in 2009.

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 1).

Table 1: 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 2). 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.

Table 2: 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 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.

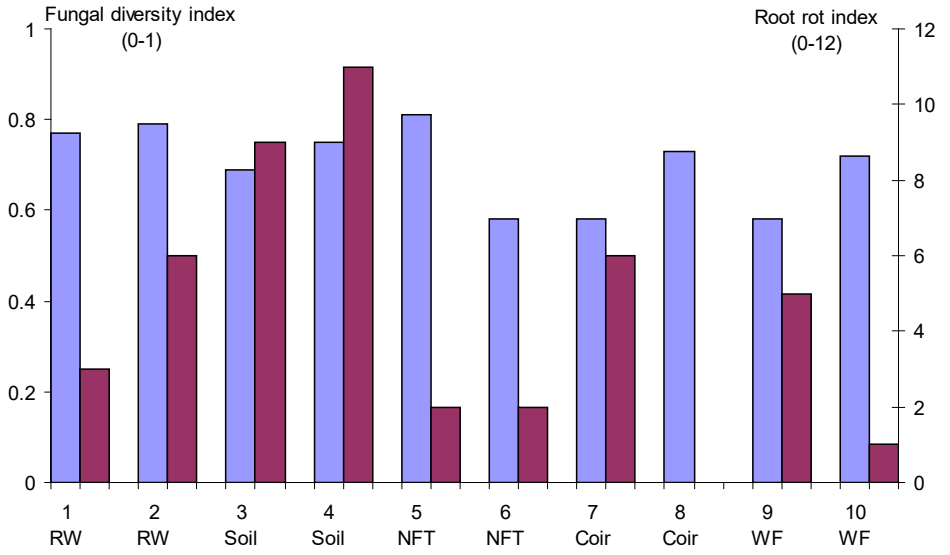

Figure 2: Fungal diversity index in August and root rot index at the end of cropping in 10 tomato crops – 2009 (RW = rockwool; WF = woodfibre)

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 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.

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.