Grower Summary

PC 281

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

Final 2011

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

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Headline

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

Background and expected deliverables

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

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

Summary of the project and main conclusions

Fungi and bacteria reported associated with tomato roots

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

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

Table 1: Fungal pathogens reported previously on tomato roots

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

Microbial populations on tomato roots in UK crops

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

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

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

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

Colletotrichum coccodes, the cause of black dot, was found in all growing media, in nine of the 20 crops and at relatively abundant levels, with increasing abundance from first fruit pick.

Traditionally considered a weak pathogen that primarily affect plants near the end of cropping, these results may indicate an increasing problem.

Species of *Pythium* and *Fusarium* were each found in most growing media, sometimes at abundant levels. Pythium root rot and Fusarium crown and root rot were obvious in some of the crops where these fungi were found. Varietal resistance, crop management and root environment likely influence why obvious root disease did not develop in the other crops.

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

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

RW – rockwool; WF – woodfibre

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

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

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

Pathogen presence and disease occurrence

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

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

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

Microbial diversity and disease prediction

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

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

Specific comparisons using T-RFLP

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

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

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

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

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

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

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

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

Potential commercial test for root micro-organisms

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

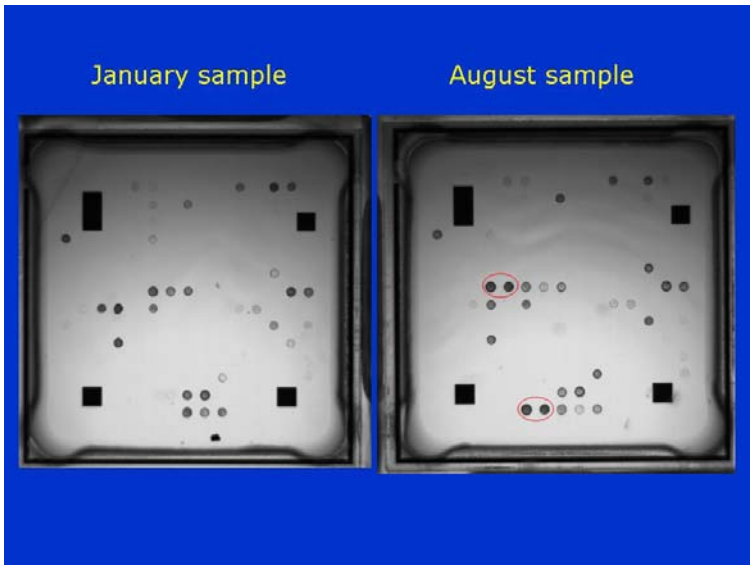

Figure 1. Tomato rhizosphere fungal microarray. DNA sequences from around 50 different fungi are on the plate, represented by 196 spots (2 sequences per fungus and 2 replicates per sequence). Colour development indicates presence of that fungus; intensity indicates relative amount. Early and late season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

Financial benefits

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

Action points for growers

- Growers should be aware of the range of diseases that can cause root loss, wilting and death of tomato plants (see Table 1).
- Growers should be aware that other potentially pathogenic fungi occur on tomato roots although their effect on plant health is uncertain. Such fungi found in this work

include *Colletotrichum acutatum, Humicola fuscoatra, Macrophomina phaseolina* and *Plectosphaerella cucumerina*; the latter fungus was found to be very common and abundant on roots.

- Growers should check roots regularly (e.g. at least every 2 weeks) for evidence of root death or disease and, where found, identify the cause. Send a sample to a diagnostic laboratory or consult a plant pathologist when the cause of damage is unclear.
- Where a root disease has caused damage on a nursery, take particular care with clean-up, disinfection and maintenance of hygiene at crop turn-around. In the work done in this project, several nurseries affected by a specific root disease one year were found to have the same root disease the next year (e.g. Fusarium wilt, Verticillium wilt, root mat, Pythium root rot).
- A microarray that can check tomato roots for a wide range of fungal pathogens and beneficial micro-organisms in a single test is being developed. Growers should be aware that it is planned to test this on commercial crops in 2012.