Horticultural Development Company

Grower summary

PC 281

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

Annual Report 2009

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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.

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Headline

A molecular fingerprinting method (T-RFLP) based on DNA fragment length is being optimised to investigate the occurrence and relative levels of microbial communities associated with tomato roots to see if root disease can be predicted. A database of fragment lengths is being established and currently contains the theoretical fragment length for 84 fungi and 3 bacteria reported associated with tomato roots. Root samples from soil, rockwool and NFT tomato crops tested using conventional methods and T-RFLP gave similar results for major culturable organisms.

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 micro-organisms in, on and around tomato roots (the rhizophere) has, until recently, been difficult and time-consuming. As a consequence, the early signs of root disease are often missed. A novel molecular method known as Terminal Restriction Fragment Length Polymorphism (T-RFLP) permits simultaneous relative quantification of micro-organisms. This 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

Fungi and bacteria reported associated with tomato roots

Examination of the scientific literature indicates a world total of at least 66 fungal pathogens (Table 1) and 75 saprotrophs that have been found associated with roots or growing media of tomato plants. A majority of these fungi occur in the UK.

Fungal genera with five or more different species causing disease of tomato roots are *Pythium* (19 species), *Phytophthora* (18), *Fusarium* (7) and *Verticillium* (5). Some fungi that affect aerial parts of tomato plants may also infect roots, including *Botrytis cinerea*, *Didymella lycopersici* and *Phytophthora infestans*. Disease symptoms caused by root-infecting fungi include root rot, foot rot, crown and root rot, damping-off and vascular wilt.

Fungal genera with five or more saprophytic species recorded associated with tomato roots or growing media are *Penicillium* (15 species) and *Aspergillus* (5). For some fungi (e.g. *Fusarium oxysporum*), both pathogenic and non-pathogenic strains are known to occur on tomato roots.

Four bacterial taxa (*Agrobacterium* spp., *Clavibacter michiganensis*, *Pseudomonas syringae* pv. *Tomato* and *Ralstonia solanacearum*) that cause disease in tomato have been recorded in tomato roots. The diseases are all rare apart from root mat disease caused by rhizogenic strains of *Agrobacterium* sp. Bacterial saprotrophs and mycorrhizal fungi and bacteria associated with tomato roots are not well documented.

Review of factors influencing tomato root diseases

Factors that influence tomato root diseases include temperature, moisture and growing medium texture, pH and nutrient levels. Effects are often complex due to interaction of factors and results are sometimes contradictory. Some reported effects on specific diseases are summarised.

Biotic factors that influence tomato root diseases include microbial amendments and organic matter amendments. Although there are numerous research reports where microbial interventions have influenced development of root disease, no microorganisms have been developed for sale as biocontrol products registered for use on tomato in the UK. For soil-grown tomato, garden waste compost is reported to reduce brown and corky root rot and a lettuce green manure crop reduced fusarium crown and root rot.

Table 1: List of fungal pathogens reported associated with tomato roots

Fungus	Disease	Comment
Alternaria solani	Damping-off	Common
Aphanomyces cladogamus	Root rot, rootlet necrosis	Uncommon
Armillaria mellea	Honey fungus	Rare
Botrytis cinerea	Grey mould	Usually on aerial pathogen
Calyptella campanula	Calyptella root rot	Rare; soil-grown crops only
Colletotrichum coccodes	Black dot	Common
Didymella lycopersici	Didymella stem rot	Uncommon on root
Fusarium species (7*)	Wilt/Crown and root rot /root rot	
Humicola fuscoatra	–	Pathogenicity disputed
Macrophomina phaseolina	Charcoal rot	Rare
Monographella cucumerina	Root rot	Minor pathogen
Phymatotrichopsis omnivora	Root rot	Not present in

		Europe
Phytophthora species (18*)	Rot rot/foot rot/damping-off	Quite common
Pyrenochaeta lycopersici	Brown and corky root rot	Common in soil crops
Pyrenochaeta terrestris	Root rot	Secondary pathogen
Pythium species (19*)	Root rot/damping-off	Common
Rhizoctonia solani	Rhizoctonia root rot	Common
Sclerotium rolfsii	Southern blight	Not in UK
Spongospora subterranea	Powdery scab	Occasional
Thielaviopsis basicola	Black root rot	Fairly common in NFT crops
Verticillium species (5*)	Verticillium wilt	–

*See Science section for full listing

Monitoring tomato rhizosphere fungi by isolation

Roots samples collected from commercial tomato crops grown in soil, rockwool and by nutrient film technique (NFT) were examined for fungi by plating onto a general nutrient agar and a *Pythium*-selective agar. The aims were: (1) to devise a root sampling procedure for each growing medium; (2) to determine if fungal taxa found associated with roots by culturing on agar were also detected by a T-RFLP test; (3) to provide cultures of a range of fungal isolates identified to genus or species level on morphological features and colony appearance, for use as reference cultures in T-RFLP tests.

For soil-grown tomato, a soil auger was used to collect soil and roots to 20 cm depth and at different locations relative to the stem base. The predominant fungi recovered on agar were *C. coccodes, Fusarium* sp., green colonies (probably *Trichoderma* spp.) and pythiaceous spp. One or more fungal colonies grew from almost all root pieces (5-10 mm in length) plated onto agar. *Fusarium* sp. was recovered more frequently from roots mid-way between propagation cubes than adjacent to cubes and vice-versa for *C. coccodes.* Pythiaceous fungi and *Trichoderma* colonies developed more commonly from

thick (5-8 mm diameter) than thin (1-1.5 mm) roots. *Fusarium oxysporum*, *Penicillium* sp. and a grey sterile fungus were recovered at a low incidence only from thin roots.

For rockwool-grown tomato, roots were obtained using a 2 cm cork borer inserted to the full depth of the slab and by cutting off roots at the slab corner. The predominant colony types recovered from roots were white (probably pythiaceous and *Fusarium* sp.), pink/red (probably *Fusarium* sp.) and *C. coccodes*. *C. coccodes* was more common on roots adjacent to the cube than mid-way between cubes or at the slab corner.

For NFT-grown tomato, a wedge of roots was cut from the channel midway between two plants. The predominant fungi recovered were *C. coccodes*, *Fusarium* sp., *Mucor/Rhizopus* and pythiaceous fungi. *Thielaviopsis basicola* was found at a low incidence. *Fusarium* sp. was recovered more frequently from brown than white roots.

For all growing media, the incidence and diversity of fungi recovered was significantly influenced by type of agar used and whether or not the roots were surface-sterilised before plating. There were also significant differences in isolation frequency between replicate plants sampled along a row, except for the NFT crop, sample position with respect to the stem base (rockwool and soil crops), root thickness (soil crop) and root colour (NFT crop).

Monitoring rhizosphere fungi and bacteria by T-RFLP

The molecular fingerprinting method T-RFLP was chosen for this study because of its simplicity and adaptability and because it detects non-culturable organisms. Using the primers and restriction enzymes detailed in this report, a database of theoretical fragment lengths of fungi and bacteria was created based on previously published data of nucleic acid sequences. So far, the database contains the theoretical fragment length for around 460 bacteria and 150 fungi. This includes 84 fungi and 3 bacteria that have previously been reported associated with tomato roots.

The theoretical length of the Terminal Restriction Fragments (TRFs) of some of these organisms was confirmed by comparison with the actual TRF obtained by T-RFLP of identified cultures (Fig. 1). To date, the TRF length of 15 fungi have been confirmed, including six pathogens (*Colletotrichum coccodes*, *Fusarium oxysporum*, *Phytophthora cryptogea*, *Plectosphaerella cucumerina*, *Pythium diclinum*, *Rhizoctonia solani*) and three saprophytes (*Cladosporium* sp., *Gliocladium* sp. and *Penicillium expansum*) reported associated with tomato roots.

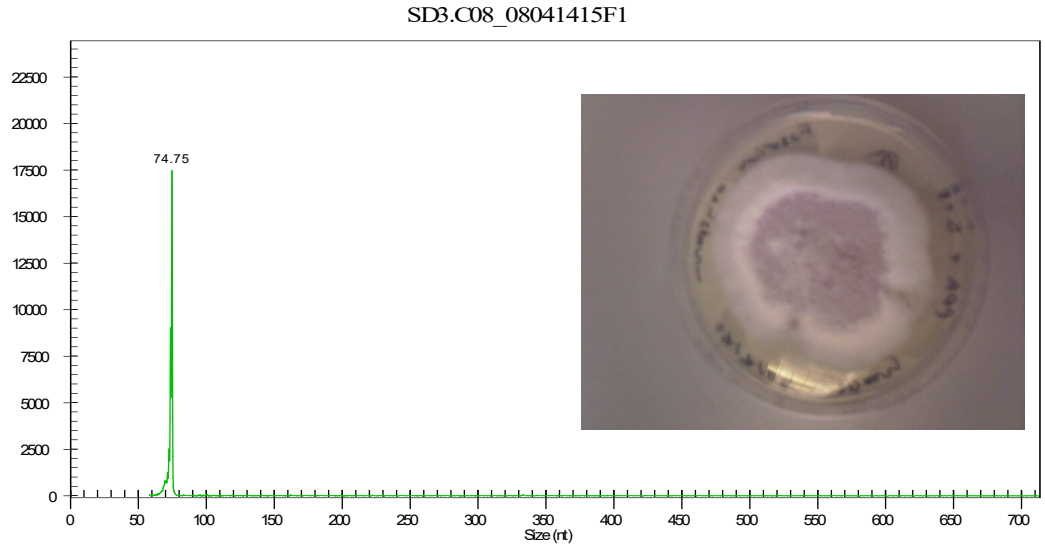


Figure 1: T-RFLP analysis of *a Fusarium oxysporum* culture, confirming the DNA fragment length when cut with a specific restriction enzyme to be 75 base pairs. A culture on agar of the fungus tested is illustrated.

Two DNA extraction methods were compared: direct extraction of c. 100 mg of tomato root and a washing method on larger root samples (<1g). The fungal fingerprints from the two extraction methods were similar; however the wash method failed to detect bacteria, and does not provide an internal plant DNA control. The direct extraction method was chosen for future work as it potentially allows semi-quantitative analysis of both fungal and bacterial genera in a single test.

Several bacterial DNA primers were examined for their consistency and ability to bind to bacterial DNA. There was some variability in the organisms detected using different primer combinations because of variability in the consensus sequences that the primers bind to in different organisms. The pair of primers that gave the most reliable and representative results, and which detected the greatest number of bacteria were selected for future work.

An examination of the effect of root thickness on microbial diversity showed that there was a greater diversity on young, actively-growing thin roots than older, woody roots of soil-grown tomato. There was also a decrease in the relative level of microorganisms, when compared with tomato DNA control, on the thicker roots (Fig. 2).

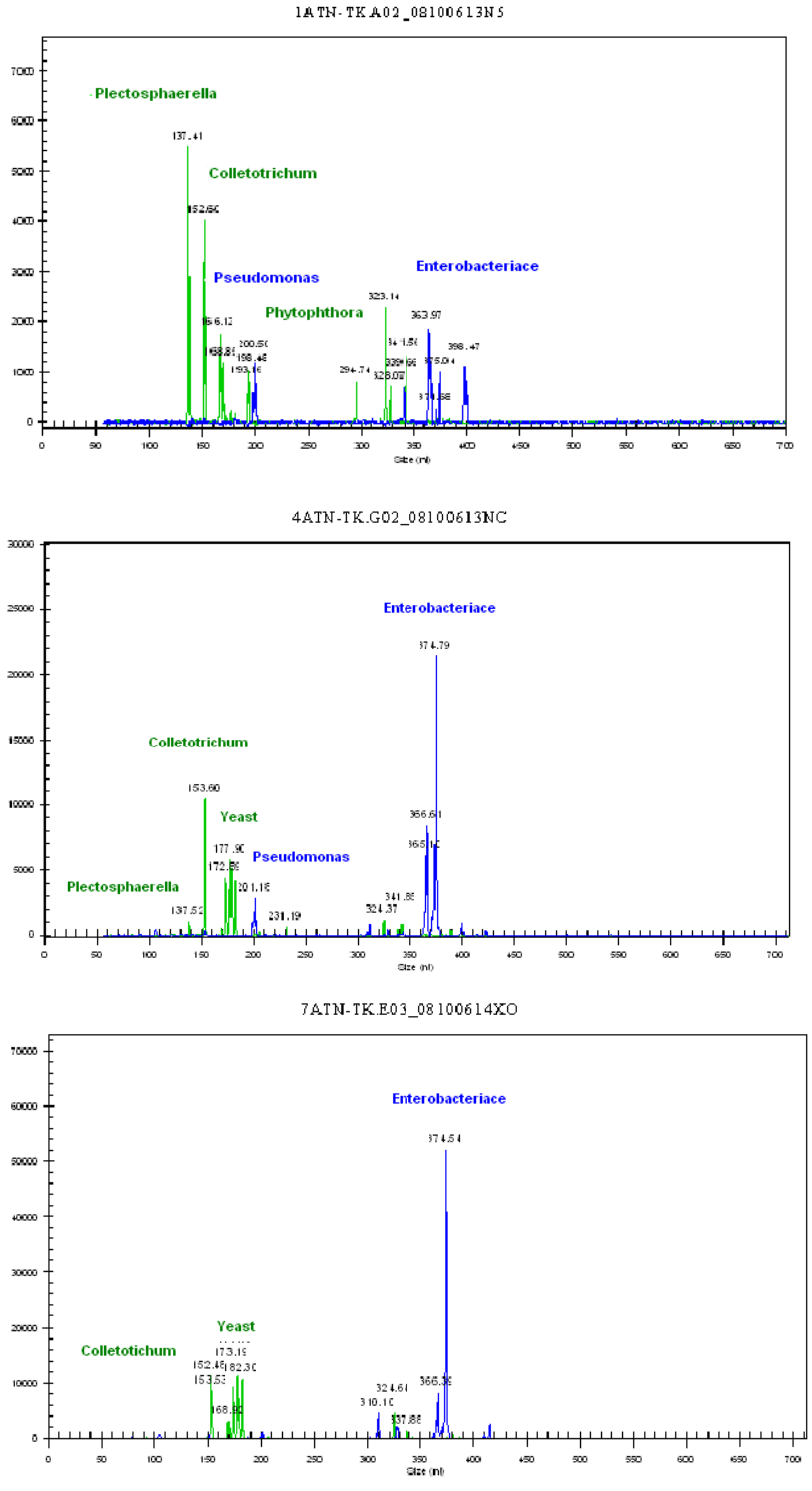
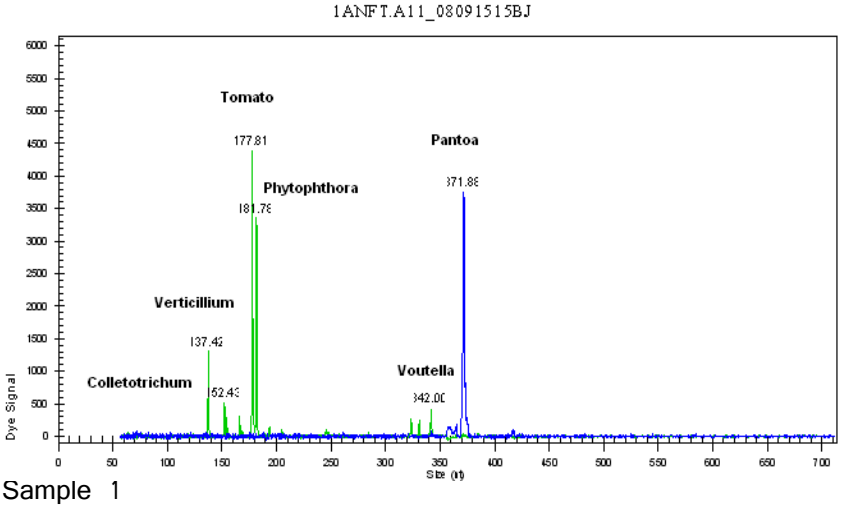


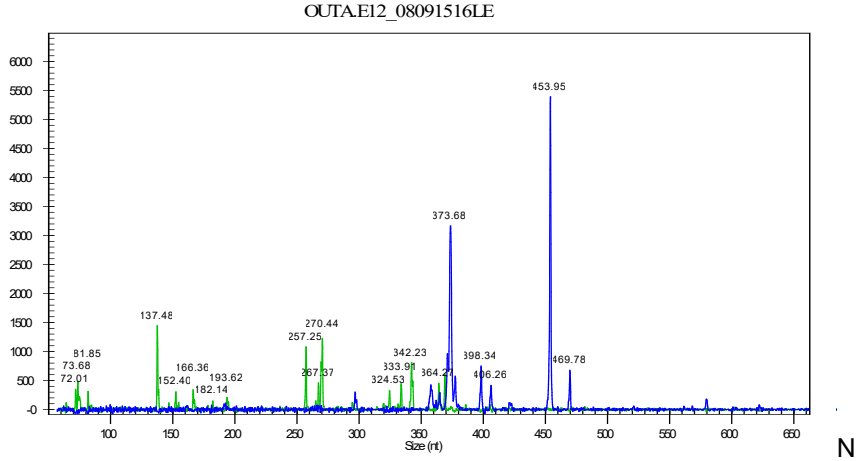
Figure 2: T-RFLP analysis of roots of different size from the same soil grown tomato plant - July 2008. Note that there are more peaks (indicating more micro-organisms) on the thin roots (top) in comparison with the medium (middle) and thick (bottom).

Three sampling methods were compared for tomatoes grown in rockwool slabs: cork-borings adjacent to the propagation cube, cork-borings mid-way between cubes and a slice of roots taken from the slab corner. There was little difference between the samples in DNA recovery or microbial diversity. Results were also very similar between three replicate plants in the same row.

Five root samples taken from one row in an NFT crop were compared. There was little variation among the root samples. There was a far greater diversity of microorganisms in the solution than in root sample (Fig 3), with little difference between inlet and outlet ends.



Sample 1 Root



NFT solution

Figure 3: T-RFLP analysis of roots and solution from an NFT crop, August 2008. Note the occurrence of *Verticillium* (confirmed in a symptomatic plant in another row) and the greater number of microorganisms present in the nutrient solution.

The range of microorganisms identified by plating onto agar and by T-RFLP was compared. Most microorganisms identified by plating onto agar were also detected by T-RFLP analysis (Table 2). A few fungi detected by plating were not detected by T-RFLP and vice-versa. This result indicates that the T-RFLP protocol being used is appropriate for studying microbial communities on tomato roots from commercial crops.

The results of T-RFLP analysis of numerous root samples show that there are many fungal and bacterial peaks present that do not correspond to TRFs on the database created. Unidentified peaks that occur frequently will be examined to determine DNA sequences; the micro-organisms will be identified by comparison with previously published sequences.

Table 2: Detection of major fungal groups and species, from roots of tomato grown in soil, rockwool and NFT, by conventional and T-RFLP methods

Fungal group or species	Detected in:		
	Soil crop	Rockwool crop	NFT crop
Colletotrichum coccodes	Both	Both	Both
Fusarium sp.	Both	Both	Both
Fusarium oxysporum	Conventional		T-RFLP
Penicillium	Both		T-RFLP
Pythiaceous	Both	Both	T-RFLP
Trichoderma	Both		T-RFLP
Verticillium			T-RFLP

Other	Both	Both	Both

Financial benefits

Root-infecting fungi are commonly found on tomato plants grown in soil, substrate and NFT crops and occasionally case 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 by prediction of the risk of root disease, 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.