Grower Summary

PC 281a

Tomato: application of next generation diagnostics for improved detection and understanding of root diseases

Final 2013

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Contractor:	ADAS UK Ltd
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Headline

- Several fungal and oomycete pathogens were commonly and consistently detected in the rhizosphere of rockwool grown crops, yet the grafted plants grew well with no obvious symptoms of root or vascular disease.
- LAMP diagnostic assays were developed for on-site rapid detection of *Botrytis cinerea*, *Colletotrichum coccodes* and *Fusarium oxysporum* in this project.

Background and expected deliverables

Root diseases pose a serious threat to tomato production with increased risk where irrigation run-off is recycled. Fungicides previously used for root disease control are no longer approved or very restricted. Growers generally wish to control root diseases without the use of fungicides. There is increasing evidence that microbial diversity on roots can benefit plant health through reducing root disease and inducing systemic resistance to some foliar pathogens. Molecular methods now provide an excellent tool for studying the largely unexplored world of root zone microorganisms. Project PC 281 using the molecular method T-RFLP revealed a tremendous diversity of microorganisms on tomato roots and variations between crops. Building on information gained in PC 281, this project aimed to apply next generation diagnostics to improve detection, understanding and control of tomato root diseases. Earlier diagnosis will permit earlier intervention. The aims of the project were: (1) to develop a laboratory based microarray for use in monitoring around 50 taxa of root microorganisms over a full season on rockwool-grown tomato and in irrigation water; (2) to utilise a portable on-site rapid diagnostic kit that is able to detect 12 microorganisms simultaneously with a high degree of specificity to supplement microarray tests to add higher specificity where required.

The expected deliverables from the project were:

1. Knowledge of when pathogenic fungi infect tomato roots and for how long they are present before symptoms occur on roots or in the crop (and whether they can occur without the occurrence of symptoms).
2. Knowledge of the combinations of pathogenic and non-pathogenic fungi and bacteria occurring on tomato roots in rockwool crops and how they change over a season.
3. A comparison of microorganism populations on tomato roots in crops grown with run-to-waste and recycled irrigation solution.

4. Knowledge of how well the microarray (used alone or with the Lamp-based diagnostic) can identify the cause of root rots and stem base vascular infections in tomato

Summary of the project and main conclusions

Objective 1 – Validate microarray for tomato root microorganisms

In a separate project (Nottingham project reference 101139) funded jointly by the University of Nottingham and Fera, a microarray has been developed for the detection of 53 fungal and oomycete species, 7 genera of fungi and oomycetes, 8 species and 6 genera of bacteria and two species and a genus of root-knot nematodes. All the microorganisms represented on the array are known to occur on tomato roots. Within this HDC project the DNA from 105 microorganisms representing 75 species and 36 genera were used to validate the array.

The primer sequences used in the array were each designed for a particular microbial species or genus known to occur on tomato roots. It is possible that the same sequence as used for one of the microarray targets may occur in a different microorganism resulting in a cross-reaction (i.e. a false positive result). The greatest level of confidence can be given in the microarray tests to those primers which were self-validated and did not show a very strong cross-reaction (Table 1).

Table 1. Microarray target organisms which were self-validated and showed little or no cross-reaction

Pathogens	Saprophytes	Bacteria
Alternaria solani	***Alternaria* spp.**	*Agrobacterium rhizogenes*
Aspergillus flavus		
Aspergillus terreus		
Colletotrichum coccodes	*Cadophora* spp.	
	Chaetomium cochliodes	*Bacillus amyloliquefaciens*
Fusarium solani	*Fusarium oxysporum*	*Bacillus subtilis*
Plectosphaerella cucumerina	***Exophiala pisciphila***	
Rhizoctonia solani	*Gliocladium roseum*	*Erwinia* sp.
Thielaviopsis basicola	***Penicillium chrysogenum***	
Verticillium dahliae	***Phoma* spp.**	***Ralstonia solanacearum***
	Trichoderma harzianum	*Rhizobium* sp.
	***Trichoderma* spp.**	

Names in bold showed no cross-reaction.

Objective 2a – Monitor rhizosphere microorganisms over a season

Occurrence of microorganisms

Rhizosphere microorganisms were monitored using a microarray every 2-3 weeks from January to October 2013 in three tomato crops grown on Maxifort rootstock on rockwool slabs. Nursery A in West Sussex grew on a run-to-waste (RTW) irrigation system; nursery B in Norfolk on a closed irrigation system with part of the drainage water recycled through a slow sand filter (pSSF); nursery C in Yorkshire on a closed irrigation system with all drainage water recycled through a Slow Sand Filter (fSSF). The number of microorganism taxa detected on roots on one or more occasion was 26, 42 and 37 at nurseries A, B and C respectively. Four potential pathogens (*Fusarium oxysporum, Fusarium redolens, Plectosphaerella cucumerina, Verticillium nigrescens*) and four saprophytic taxa (*Aspergillus flavus, Olpidium brassicae, Penicillium* spp., *Petriella asymmetrica*) were detected at relatively high abundance in all crops and on most samples. *Colletotrichum coccodes* (cause of black dot) and *Rhizoctonia solani* were commonly detected only at nursery B; *Verticillium albo-atrum* was commonly detected only at nursery C. Eight *Pythium* spp. were detected with four of them (*P. diclinum, P. irregulare, P. myriotylum, P. paroencandrum*) common in all crops. The detection of some of the potential pathogens on roots in rockwool crops was unexpected: *Colletotrichum acutatum, Rhizoctonia solani, Pyrenochaeta lycopersici*. Examination of the data across all crops showed the fungal and bacterial species diversity on roots declined as the season progressed whereas that of oomycetes increased. There was no evidence of leaf yellowing, plant wilting or plant death due to root disease despite the occurrence of several potential pathogens on roots for many weeks. These results suggest that tomato crops grown on Maxifort rootstock on rockwool slabs can tolerate a certain level of one or more root pathogens for a considerable time without evident adverse effect on crop growth. Possibly the inoculum level of pathogens was insufficient to cause serious root damage; and/or rootstocks were able to rapidly produce new roots to compensate for diseased roots.

Comparison of irrigation systems

The three irrigation systems represented by the three nurseries in this study appeared to have an effect on rhizosphere microorganisms present at those sites. However, it is important to note that some of the differences may be explained by site differences other than irrigation system. Nursery A irrigation water was run-to-waste (RTW) and roots from this crop had significantly less species diversity across the year compared to the other two systems. A significant difference in the mean species diversities was also found between the pSSF and fSSF systems for both the combined (all microorganisms) and fungal diversities

from the root samples. Roots sampled from Nurseries B (pSSF) and C (fSSF) generally held a greater number of taxa, with the full SSF system having fewer rhizosphere microorganisms. Rhizosphere microorganisms were monitored every 2 months at two additional nurseries, where the recycled water was treated by UV (D) or heat (E). These two nurseries were both growing on the rootstock Emperador, rather than Maxifort. Differences in fungi and oomycetes were more pronounced in the treated solution sampled than in the rhizosphere itself, suggesting that though treating irrigation water removes a number of pathogenic species, its recycling through the crop allows them to be picked up again. There were no significant differences in levels of bacteria detected between the two treatment systems.

Objective 2b – Investigate tomato root diseases by microarray and conventional tests

Work on this aspect was limited by the lack of root disease problems in rockwool crops in 2013, probably due in part to the switch by most growers to growing on rootstocks. Use of the microarray detected more potential pathogens than conventional tests. There was little difference in the number and level of potential pathogens as determined by microarray between 'poor' plants and 'good' plants in the same area. Root disease problems were generally associated with a complex of pathogens; diseased roots generally had a lower microbial diversity than 'good' roots.

Objective 3 – Examine microorganism population changes with reference to root zone physical environment

The root zone environment physical factors (pH, EC, temperature) measured by the three host nurseries remained within acceptable range throughout crop production. The rhizosphere microbial populations at each site were relatively stable throughout crop production with respect to species diversity. There was no obvious crop production or rhizosphere physical factor to hypothesise as a cause of a reduced species diversity at all three sites in February; possibly the fall in species diversity at this time was an artefact introduced during laboratory tests. Specific experiments designed to alter one physical factor, for a defined period, offer better prospects for investigating the effect of root zone physical factors on rhizosphere microbial populations.

Objective 4 – Develop and validate LAMP assays to discriminate closely related taxa

Potential targets for in-house point-of-care diagnosis were first identified during meetings with tomato growers. Real-time LAMP assays, using the Genie II (Optigene UK) portable

Lamp machine were then developed and validated for *Botrytis cinerea* (grey mould), *Colletotrichum coccodes* and *Fusarium oxysporum*. The assays were then validated using DNA from closely related microorganisms and those found to be common on tomato roots. The tests gave positive results on tomato leaf and root samples naturally infected with these fungi. The assays therefore represent point-of-care tests for these pathogens that can be used to provide positive identifications within 30 minutes of testing.

Financial Benefits

Root diseases of tomato are numerous, widespread and potentially devastating. Plant losses due to root disease have cost over £50,000 on at least two nurseries in recent years. There may be potential yield loss occurring on many nurseries due to root death from low levels of disease. At least 66 fungal pathogens and four bacterial pathogens have been found associated with roots or growing media of tomato plants around the world. In the UK, the most common root diseases are Pythium root rot, Phytophthora root and stem base rot, corky root rot, black dot root rot, Fusarium wilt, Fusarium crown and root rot and Verticillium wilt; a range of other diseases occur from time to time. Root diseases are generally difficult to diagnose as the range of symptoms is limited, symptoms can overlap, plants can be infected by several pathogens simultaneously and some causal microorganisms are difficult to isolate.

Non-pathogenic fungi and bacteria also occur in the root environment and can influence occurrence of root diseases. Obtaining good information on the occurrence and levels of rhizosphere microorganisms, either pathogens or saprophytes, has, until the recent advent of molecular methods, been difficult, time-consuming and relatively expensive.

Action points for growers

There are no immediate action points. However, there are several points of interest arising from this project which growers should note with regard to detection and control of root diseases.

1. The use of grafted tomato plants appears to reduce greatly the risk of severe root disease. We consistently detected several plant pathogens in the roots of Maxifort and Emperador and yet the crops grew and yielded well, with no yellowing, wilting or stunted growth.
2. Tomato plants can have a diverse microbial population on roots, including potential pathogens, even before they are planted onto slabs.

3. Rhizosphere microbial diversity was greater on plants grown with recirculation of the nutrient solution than with the solution run-to-waste. It is an ecological principle that a diverse community is likely to be more resistant to change than a simple community.
4. Although many microorganisms species are present on tomato roots at planting and persist throughout cropping, additional species, including pathogens, (e.g. *Colletotrichum coccodes*; *Verticillium albo-atrum*) may occur during crop production. This indicates a potential benefit from maintaining disease precautions during crop production.
5. *Colletotrichum acutatum*, which is usually reported as a fruit pathogen, was commonly detected on roots. Identification was confirmed by barcoding. Possibly this fungus can also infect and damage roots.
6. *Plectosphaeralla cucumerina* (*Fusarium tabacinum*) was commonly and consistently detected on tomato roots in this project. Previous work indicates this fungus is common in hydroponic crop production. Although generally regarded as a weak pathogen, there is a recent report from South Korea of the fungus causing wilting and death of tomato. Growers should be alert to any reports of *Plectosphaerella* associated with a tomato disease in Europe.
7. Nurseries can differ in the range of pathogens commonly found on tomato roots. There is evidence elsewhere that some pathogens (e.g. *Verticillium albo-atrum*) can persist on a nursery after an outbreak of Verticillium wilt.
8. The detection of several potential pathogens on roots from when plants are planted on slabs, the continued occurrence of these microorganisms through cropping, and the lack of any visible deleterious effect on crop growth at any of the monitored sites, raise the question of how important is hygiene with regard to control of root-infecting pathogens when grafted plants are grown on rockwool slabs? For example, are there potential savings from a reduction in some aspects of hygiene measures between crops, and from treating re-cycled water during cropping? However, until there is experience over several seasons of growing grafted plants on rockwool slabs, it is recommended that between-crop hygiene and water treatment are maintained as precautionary measures against damaging root disease.
9. A microarray for detection of tomato rhizosphere microorganisms has been validated and can be used for investigation of root diseases. Eighteen of the probes on the array are species-specific, were self-validated and showed no or low-level cross-hybridisation with other species. These comprise 12 fungi and oomycetes (including *Plectosphaerella cucumerina*, *Fusarium oxysporum, Fusarium solani*, *Rhizoctonia*

solani, *Thielaviopsis basicola*, *Trichoderma harzianum* and *Verticillium dahliae*) and six bacteria (including *Bacillus amyloliquefaciens*, *Bacillus subtilis* and *Ralstonia solanacearum*). The usefulness of the microarray would be enhanced by increasing the number of probes with nil or low level cross-reaction.

10. 'LAMP' diagnostic assays have been developed in this project that permit very accurate, sensitive and rapid on-site tests for three tomato pathogens: *Botrytis cinerea*, *Colletotrichum coccodes*, *Fusarium oxysporum*. Assays have been developed elsewhere for *Pepino mosaic virus* (3 strains) and *Pythium aphanidermatum*. Growers should be alert for laboratories (e.g. Fera) or commercial companies (e.g. Optigene) offering these as chargeable tests.