Project title:	Protected tomato: Evaluation of biological treatments, biocides and an improved diagnostic for control of root mat disease
Project number:	PE 029
Project leader:	Sarah Mayne, ADAS
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Previous report:	None
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Location of project:	ADAS Boxworth & commercial sites
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations. Growers should note that this trial included substances which are not registered as crop protection product in the UK. Only products officially approved as plant protection products should be applied to control pest, disease and weed problems or used as plant growth regulators. Before using any such substance growers should refer to product approval and label documents and seek guidance from a BASIS qualified consultant.

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headlines

- The most effective products to reduce the symptoms of root mat in rockwool were Carbon Gold biology blend and a mixture of Serenade ASO and Trianum P
- A molecular diagnostic has been validated to identify transformed tomato roots, even in the absence of the initial bacterium that infected the plant

Background

Root mat disease in tomato was first observed in 1999 on a batch of plants propagated in the Netherlands. The disease was confirmed in 2000 when symptoms were shown to be caused by *Rhizobium radiobacter* (previously *Agrobacterium* bv. 1) harbouring a root-inducing (pRi) plasmid. A piece of this plasmid (T-DNA) is transferred from the bacterium during root infection where it is incorporated into the plant cell genome. Genes contained on the plasmid are expressed when inserted into the plant genome leading to a plant hormone imbalance that results in root proliferation. Subsequent investigation showed that the same plasmid could potentially be harboured by a number of other bacteria, including members of the genera *Ochrobactrum*, *Rhizobium* and *Sinorhizobium,* which were also able to induce symptoms of root mat in tomato and cucumber. In the related disease crown gall, caused by a tumour-inducing plasmid (pTi), it has recently been shown that various *Agrobacterium* and *Rhizobium* species are associated with the disease in raspberry. The predominant symptom in tomato is extensive root proliferation within the propagation cube and across the slab surface. Roots grow upwards out of the top of the propagation cube, commonly around the irrigation peg, and within the cube and slab causing swelling and distortion. Drainage channels may become blocked by the excessive root growth.

A partially selective bacterial growth medium (Schroth's medium) is available to isolate, identify and quantify *R. radiobacter* but does not distinguish pathogenic isolates with root inducing plasmids. Non-pathogenic strains of *R. radiobacter* are ubiquitous in soils, circulating liquid nutrient media and associated plant material. A qPCR test is available to determine if *R. radiobacter* isolates contain Ri plasmids associated with root mat. However, not all variants of this plasmid are detected using the q-PCR assay. It is therefore unclear whether the assay can be reliably used to detect plasmid DNA incorporated into transformed roots of tomato and cucumber plants, where the rhizogenic bacteria may no longer be present, before symptoms of root mat have developed. The availability of a reliable qPCR test able to detect the known diversity of Ri plasmids would both permit accurate evaluation of infection (including pre-

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symptomatic) and strengthen reliability of results from work investigating efficacy of control measures. Further sequence analysis of plasmids from different isolates and full test validation for detection of transformed tomato root tissues is required. Such a test would allow better determination of when infection occurs during plant growth.

During 2016 a survey of UK tomato grower's experiences with root mat was carried out. This showed that 88% of growers surveyed had experienced root mat on their nursery. Most estimates for the % of crop affected in the 2015 season were at 1-5%, but one grower reported an incidence of more than 50%. Of the growers that had experienced root mat on their nursery, all described symptoms as either moderate or severe (none reported only slight symptoms). 67% of growers reported removing the plastic wrappers from slabs in an effort to control the disease. Using managed irrigation or any biological products were less popular control options. Estimated efficacy of these methods varied largely between respondents. Some growers also felt some varieties of scion, or rootstock/scion combinations were more susceptible. Overall, the impacts of irrigation, subsequent drainage and substrate aeration were considered important by the growers questioned. There was also a suggestion that light levels may play a role in symptom expression.

Summary

This project focusses on control of root mat by both prevention of infection and reduction in subsequent symptoms. In the first year of the project current knowledge has been reviewed (Objective 1), an improved diagnostic test has been developed (Objective 2), and the efficacy of a number of biocontrol products has been examined in trials at ADAS Boxworth (contributing to Objectives 3 & 4). The project's specific aims and objectives for this three year project are summarised below.

(i) Project aim(s):

To identify biological treatments and biocides that reliably control or suppress root mat disease by prevention of infection and transformation of protected tomato by bacteria carrying the root initiation plasmid (pRi) and to develop a rapid molecular test for early detection of infected plants.

The results of work carried out in 2016 are summarised below by the specific objective addressed.

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Objective 1 - To review and summarise current knowledge of root mat disease in tomato and cucumber through production of text and photographs for an AHDB Factsheet/review document.

The review can be accessed in its entirety via the AHDB Horticulture website, but key findings are summarised below.

- Root mat in tomato is caused by rhizogenic plasmids (pRi), and crown gall is caused by tumorigenic plasmids (pTi), most commonly vectored by *Rhizobium radiobacter*, a common soilborne bacterium.
- Bacteria causing root mat and crown gall may both acquire and lose these plasmids.
- Recently, the genus *Rhizobium* was revised to incorporate all species previously described as *Agrobacterium*. This classification was based on 16S ribosomal DNA analysis and hence genetic relatedness.
- The development of crown gall (and also likely root mat) is activated by fresh wounds on roots or stems which produce exudates that act as signal molecules; bacteria move to the wound site along the chemical gradient.
- Infection occurs when a piece of the plasmid DNA, known as the transferred DNA (T-DNA), is transferred from the bacterium and incorporated into the host plant nuclear DNA.
- Genes contained on the plasmid are expressed when inserted into the plant genome leading to a plant hormone imbalance that results in uncontrolled root proliferation (root mat) or tumour growth (crown gall) at the infection site.
- Infected plant cells synthesise simple novel metabolites, known as opines, that are not found in normal plant tissues. The pattern of opines synthesised is determined by the type of virulence plasmid in the bacterium and, in general, the virulence plasmids also confer on the infecting bacterium the ability to utilise the same opines as nutrients.
- In inoculation experiments, both inoculum concentration and plant age have been found to influence infection success and severity of symptoms.
- Substrate type has also been found to affect root mat and both incidence, and severity of symptoms has been observed to differ between different types of coir.
- Once a plant is infected with the Ri or Ti plasmid, there are no known treatments which will prevent symptom development. Consequently, the current focus for control of both root mat and crown gall disease is to prevent infection. As with other plant diseases, this may be achieved by host resistance, by environment manipulation to make conditions unfavourable for infection, or by reduction/elimination of rhizogenic *R. radiobacter* inoculum in the environment around plants.
- Most of the tomato varieties and rootstocks currently grown in the UK appear to be susceptible to root mat. Cultivar resistance to crown gall has been reported (e.g. in rose)

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and one tomato variety (cv. Kanavaro) has been observed to be less susceptible to root mat than others.

- No root zone environment manipulation treatments that reliably reduce root mat have yet been identified. There is speculation that oxygen level in irrigation solution and irrigation frequency may influence the disease.
- There is good reason to believe biological treatments could reduce tomato root mat by influencing the population of rhizogenic bacteria around tomato roots.
- Specifically, recent work on crown gall disease showed that a quorum sensing signal is produced by populations of *A. tumefaciens* that controls transfer of the Ti plasmid. Transfer of the Ti plasmid only occurs at high population densities of *A. tumefaciens*, when concentration of the signalling molecule is high.
- Various isolates of *Bacillus*, *Pseudomonas* and *Trichoderma* species have been shown to reduce crown gall, possibly through reduction of *A. tumefaciens* populations. Assuming quorum sensing also operates with root mat disease, biological products might reduce root mat if they prevent the population reaching a threshold concentration where plasmid transfer occurs.
- Modified strains of *Agrobacterium* have shown most promise in control of crown gall and some (e.g. Galltrol) are marketed for this purpose, although not in the UK.
- Previous trials with biological products for control of root mat were largely unsuccessful due to low incidence and/or high variation in disease occurrence.

Objective 2 - To develop and fully validate a rapid molecular test for detection of T-DNA from different Ri plasmids in tomato roots prior to symptom occurrence

A collection was made of 68 isolates of *Rhizobium* from UK tomato and cucumber crops with bacterial root mat and additional reference strains known to cause similar root proliferation in different crops around the world. Whole genome sequencing of each isolate, in conjuction with pathogenicity testing on tomato seedlings in the greenhouse, confirmed all those able to cause root mat (rhizogenic) on tomato or cucumber as *Rhizobium radiobacter* carrying a particular root inducing (Ri) plasmid, known as a cucumopine Ri plasmid. Not all isolates from tomato or cucumber with root mat were pathogenic and all non-pathogenic isolates lacked the Ri plasmid. All of the reference isolates causing root proliferation in other crops carried a different Ri plasmid to the cucumopine plasmid and were identified as other *Rhizobium* species (*R. vitis* and *R. rhizogenes*).

Genome analysis of the rhizogenic tomato and cucumber isolates confirmed that they could be specifically detected using existing polymerase chain reaction (PCR) and quantitative PCR

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(qPCR) methods that target transfer-DNA (T-DNA) that is exchanged between the *R. radiobacter* Ri plasmid and the plant genome after bacterial infection. A new DNA extraction method was developed to allow direct detection of the T-DNA sequences in plant roots. This was compared with an existing test that first involves a 48 hour enrichment of *R. radiobacter* in selective media prior to its detection by the PCR methods. Both methods were able to detect the T-DNA target sequences in infected tomato plant roots, even before symptoms developed in inoculated plants. It is hoped that the direct DNA extraction from tomato roots will permit testing of young propagation material to allow screening for infection by rhizogenic *R. radiobacter*, even in the absence of the bacterium that caused the original infection, prior to transplanting for commercial production.

Objective 3 - To quantify the effect of biological-based products applied during propagation on infection and transformation of roots and incidence and severity of root mat disease

In 2016, a preliminary trial was set up to establish an effective inoculation method. Plants were grown in rockwool propagation cubes held in open trays to create a continually damp root environment. Symptoms were produced successfully in tomatoes of variety Elegance, both ungrafted and grafted onto Emperador rootstocks (Figure 1). Infection occurred in both plants inoculated at the plug stage, at 19 days old, following wounding by rough handling of plugs at transplant. Symptoms were also produced in seedlings that were inoculated two weeks after, at 33 days old, following root wounding using a scalpel.

Infection was also observed in control plants that had not been inoculated, likely due to spread by water splash or possibly insects. Spare plants kept in a separate greenhouse never exhibited symptoms of root mat. Samples of plant roots sent for testing at Fera using the assay described in Objective 2, confirmed that T-DNA was present in all treatments.

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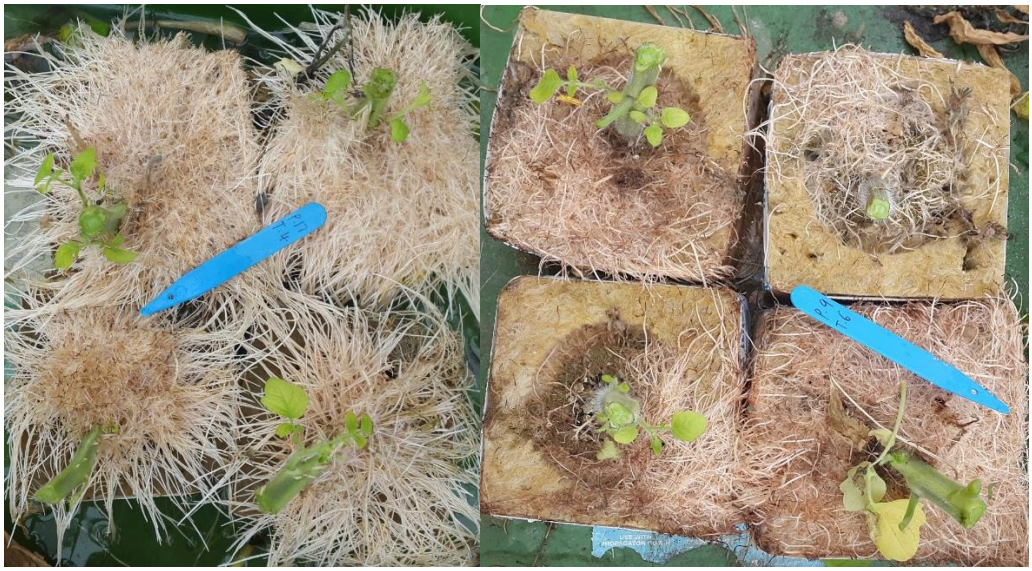

Figure 1. Representative examples of severe symptoms observed in ungrafted (left) and grafted (right) plants - ADAS Boxworth, 2016

Following this trial, ungrafted Elegance was selected for use in a larger trial to test a variety of non-conventional products for their ability to control root mat on tomato. This trial was set up using commercial rockwool slabs, with plants grown on for 14 weeks after inoculation. Each plot contained six plants, and the treatments applied are summarised in Table 1.

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Table 1. A summary of the treatments, largely biological, applied to plots for control of root mat - ADAS Boxworth, 2016

Treatment	**Product**	**a.i.**
1	Untreated uninoculated	water
2	Untreated inoculated	water
3	Unwounded inoculated	water
4	Trianum P	*Trichoderma harzianum* T-22
5	ProParva	Plant auxins
6	Jet 5	Hydrogen peroxide
7	Proradix	*Pseudomonas* sp. DSMZ 13134
8	Serenade ASO	*Bacillus subtilis* QST 713
9	Carbon Gold Biology Blend	Enriched biochar, microbes, wormcasts, seaweed etc.
10	Trianum P Serenade ASO	Combination of these 2 treatments
11	Additional booster treatment of T10 24 hours after inoculation	Combination of these 2 treatments

The proportion of the rockwool cube surface affected by root mat was assessed throughout the course of the trial. By the final assessment, on 10 November 2016, the majority of plants were expressing symptoms. It is likely that no treatments used alone are capable of preventing infection entirely, though some were observed to suppress symptom expression. It should also be noted that the plants with the most obvious root mat on the cube surface were not always the most badly affected when the final, destructive assessments were carried out. Proradix treatment (a *Pseudomonas* sp. shown to effectively colonise solanaceous root systems) resulted in the lowest incidence of visible infection on the cube surface covered by the end of the trial, but on closer inspection, plants with the healthiest root systems were those in plots treated with Carbon Gold. Treatment 10, a multi species inoculation, also seemed to have an effect, and a repeated treatment following inoculation (Treatment 11) improved this control. Differences between Treatment 10 and 11 were not statistically significant, but there was a conserved trend for lower incidence and severity in Treatment 11. At the final assessment, there were statistically significant differences between treatments recorded for cube and slab severity scores, and % of the slab surface affected. There were not significant differences between treatments in terms of % cube surface affected (Figures 2 and 3).

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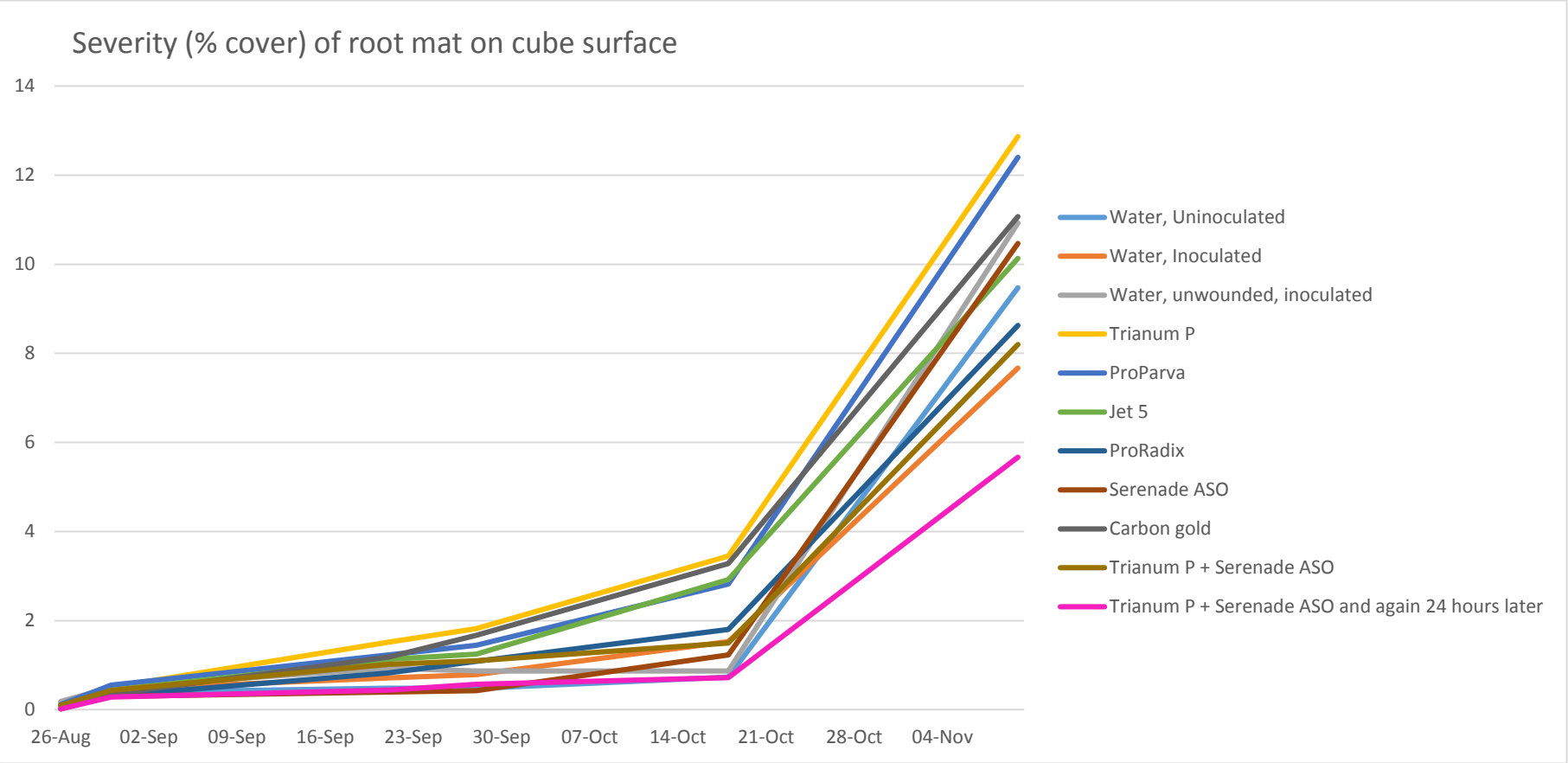

Figure 2. Severity, assessed as % cover of the cube surface, following treatment and inoculation - ADAS Boxworth, 2016

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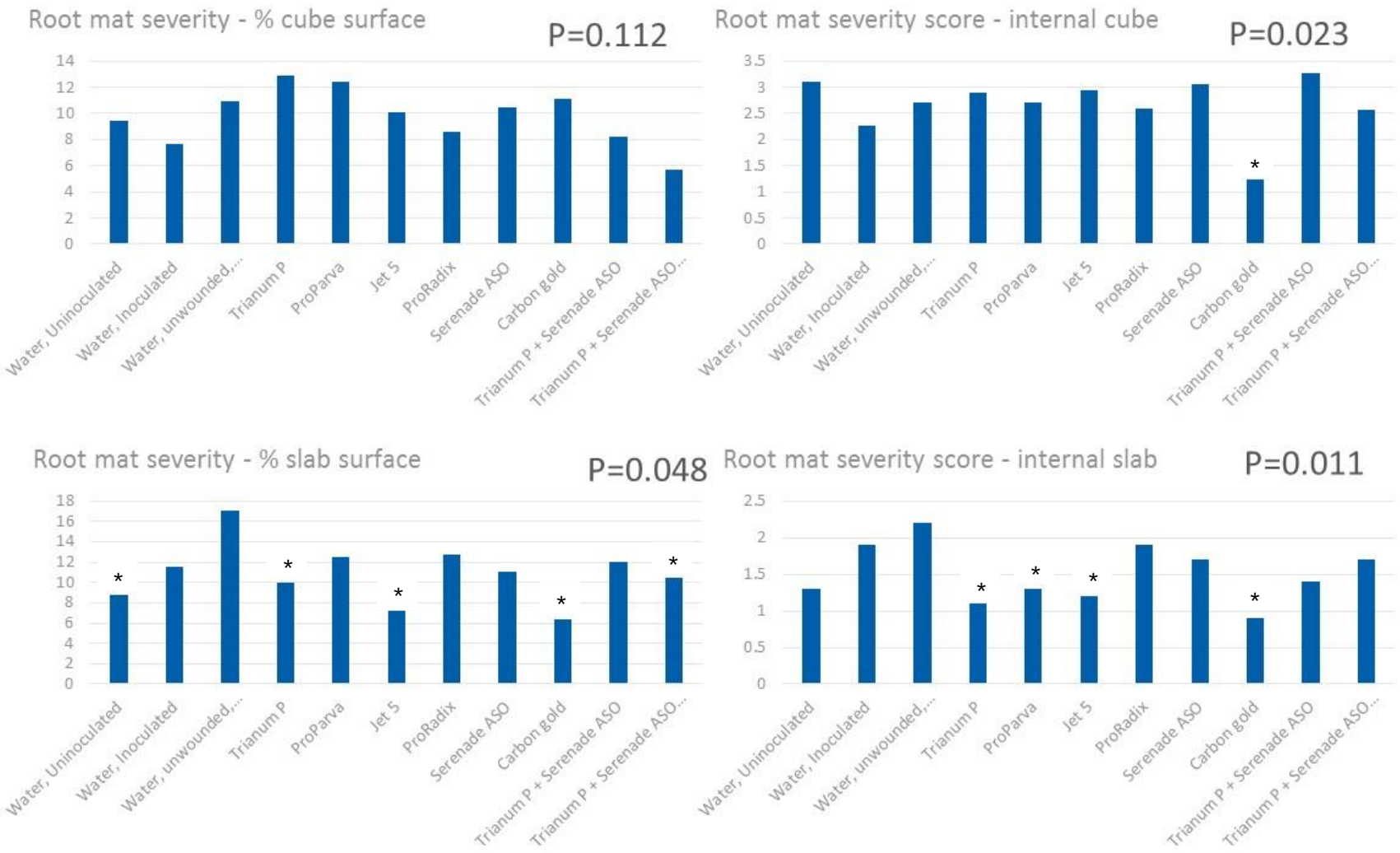

Figure 3. A summary of root mat symptom expression in rockwool cubes and slabs at the final assessment, 14 weeks after inoculation - ADAS Boxworth, 2016. Treatments significantly reduced compared with inoculated unwounded control (T3).

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Samples of plant roots were sent for testing at Fera using the assay described in Objective 2, confirming that T-DNA was present in all treatments.

Commercial trials beginning in 2017 are set to further examine Objective 3 (product efficacy), and will also focus on Objective 4 (post-planting treatments). The treatments selected, based on 2016 trials, are Carbon Gold and a mixed isolate product (as drenching with Serenade ASO is not approved for commercial use). Work contributing to Objective 5 (biocides) will begin at clean-up at the end of the 2017 season. Work contributing to Objective 6 (technology transfer) is ongoing.

Additional observations

During Year 1 of this project, it came to our attention that a grower in the USA was also experiencing problems with root mat, in both tomatoes and cucumbers. As such, we advised the set-up of a large replicated trial on a commercial nursery to examine the effect of removing propagation cube wrappers and/or the use of a drench of Prestop during propagation. This trial was assessed twice in autumn 2016, but statistically significant differences in root mat incidence and severity were not observed on either occasion. Root mat developed to a high incidence (almost 100%) and moderate severity (a mean of 3.2 on a 0-5 scale) in all treatments at 8 weeks after planting. Neither treatment reduced the incidence or severity of the disease.

Further to this, the effect of different slab substrate types on root mat was observed on a UK site where multiple coir mixes are used in slabs. This site was assessed in late 2015, before the start of this project, and again in late 2016. On both occasions, a coir mix which reportedly allowed better drainage and aeration appeared to result in less severe root mat developing (i.e. a greater chip to pith ratio). It must be noted that these observations are based on natural infection and treatments were on single large blocks, not replicated blocks in a randomised trial design.

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Financial Benefits

- Consequential losses and additional costs due to the presence of root mat disease on one 26ha UK nursery are estimated at around £0.75 million per year, averaging £29 000/ha/year.
- Financial losses arise due to increased costs of crop management, an increased proportion of fruit being out of specification, and an increased susceptibility of transformed plants to secondary root diseases.
- As root mat does not commonly affect all plants in a crop evenly, crop steering becomes increasingly difficult as symptoms appear and the previously homogenous crop profile becomes randomly variable.

Action Points

- Any product applications designed to prevent infection, spread or development of symptoms of root mat should begin at the earliest stage possible e.g. at sowing or in propagation
- Though research is at an early stage, it appears that repeated applications of products containing more than one beneficial organism are more effective than products containing only a single strain
- As *R. radiobacter* is ubiquitous in the environment, good hygiene and sanitation practices should be followed throughout the year
- Monitoring when and where symptoms occur each year may help identify areas where more effective clean-up is required
- Testing of propagation material before transplanting may help prevent introduction of infection; the assay tested in this project will be further examined in 2017.
- Reducing initial inoculum concentration of *R. radiobacter* resulted in slower development of root mat. Therefore treatments that suppress pathogen populations are likely to delay or prevent disease development. However, once established, experience shows that root mat can spread quite readily from infected to healthy young plants.
- Carrying out a strict clean-up protocol at crop turnaround is considered ‘best practice’ and will help ensure *R. radiobacter* inoculum is eradicated or reduced - this can include the cleaning of irrigation lines with the aim to clear biofilms that have built up over the year. Biofilms have been shown to harbour *R. radiobacter* and could initiate infection of new crops on site each year.

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SCIENCE SECTION

Introduction

Root mat disease of tomato caused by strains of *Rhizobium radiobacter* carrying a root-inducing (Ri) plasmid is an increasing problem in the UK and elsewhere. Current knowledge of disease biology and crop observations both indicate that infection probably occurs when plants are young, including during propagation, though symptoms can take many weeks to develop. The disease causes excessive vegetative growth, reduced fruit size and quality and secondary root rots. Together these result in significant crop losses estimated at 15% and additional management costs.

There are no proven treatments for disease control. Current efforts focus on biological treatments, crop management and hygiene; there are no approved bactericides. An increasing number of biological products reported to increase plant health and/or resistance to disease are now available; the NatuGro programme, for example, is used quite widely although there is no evidence for effectiveness against root mat.

A real-time PCR assay previously developed detects the pRi plasmid in isolates of *Rhizobium radiobacter.* It is unclear whether the assay detects all variants of pRi or can be reliably used to detect transformed roots before symptoms of root mat have developed. The availability of a reliable test that detects pRi in plants as well as in bacteria will allow accurate early detection of infected plants and more reliable evaluation of control measures.

The project's specific aims and objectives are summarised below.

(ii) Project aim(s):

To identify biological treatments and biocides that reliably control or suppress root mat disease by prevention of infection and transformation of protected tomato by bacteria carrying the root initiation plasmid (pRi) and to develop a rapid molecular test for early detection of infected plants.

(iii) Project objective(s):

1. To review and summarise current knowledge of root mat disease in tomato and cucumber through production of text and photographs for an HDC Factsheet.
2. To develop and fully validate a rapid molecular test for detection of T-DNA from different Ri plasmids in tomato roots prior to symptom occurrence;
3. To quantify the effect of biological-based products applied during propagation on infection and transformation of roots and incidence and severity of root mat disease;

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4. To evaluate the effect of biological-based products applied after planting on infection and transformation of roots and incidence and severity of root mat disease;

5. To determine the efficacy of some biocides used at crop turnaround in reduction of *Rhizobium* populations and Ri plasmid;

6. To transfer knowledge to growers through articles, presentations, on-site visits and project reports.

The review produced for Objective 1 of this work can be accessed as a separate document, available from AHDB Horticulture. Its key findings are summarised in the Grower Summary and in the Results section of this report.

Materials and methods

Objective 1 - To review and summarise current knowledge of root mat disease in tomato and cucumber through production of text and photographs for an HDC Factsheet and a review document.

In early 2016, information from a variety of sources on root mat was collated. This included information in academic literature, in past AHDB Horticulture/HDC projects and technical information provided by the horticulture industry. The document covers aspects of the biology of *R. radiobacter* and the Ri plasmid relating to the control of the resulting root mat disorder.

Objective 2 - To develop and fully validate a rapid molecular test for detection of T-DNA from different Ri plasmids in tomato roots prior to symptom occurrence

Collection of isolates

A diverse collection of 68 *Rhizobium* isolates obtained from tomato and cucumber with root mat as well as various other host plants with root proliferation was assembled. This included cucumber isolates dating back to the first root mat outbreaks in 1974 and cucumber and tomato isolates from root mat outbreaks studied in former projects (PC 149 and PC 241) as well as newly collected isolates from diseased tomatoes sampled in 2015. All isolates were tested for presence of the Ri (root-inducing) plasmid using a conventional PCR assay (Haas *et al*., 1995) and a TaqMan qPCR assay (Weller and Stead, 2002).

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Pathogenicity testing of isolates

Pathogenicity was established on a selection of the isolates with and without Ri plasmids by inoculation of the roots of tomato seedlings cv. Moneymaker (at 3-4 leaf stage) growing in John Innes #2 compost. Inoculation was performed by cutting through the soil and roots to one side of each plant with a sterile scalpel and pouring 15 ml of an aqueous suspension containing $1x10^8$ cfu (colony-forming units) per ml of each isolate into the cut. Control plants were inoculated in the same way but with sterile water. Plants were grown on at a constant 25°C, 14 hour daylength and average 70% RH and development of root mat symptoms was observed over a 5 week period.

Genome sequencing of isolates

Whole genome sequencing was performed on each isolate using the Illumina Miseq next generation sequencing platform. Contigs for each isolate were then aligned and compared to allow phylogenetic analysis of relative sequence similarity between each isolate. Sequences were identified that corresponded to known T-DNA regions within each Ri plasmid that are amplified by the primers of Haas *et al*. (1995) and Weller and Stead (2002).

Development of assay for detection of T-DNA in transformed tomato plants

A method was evaluated for extraction of DNA directly from tomato roots for subsequent detection of Ri plasmid T-DNA sequence using qPCR. This involved randomly sampling 1-2g of root tissue per seedling and freezing in liquid nitrogen followed by grinding with CTAB (cetyltrimethylammonium bromide) and automated purification and magnetic capture of the DNA using Promega Wizard® Magnesil paramagenetic particles.

The new test method was compared with the established test (Weller and Stead, 2002) that involves pre-enrichment, prior to testing, of any *Rhizobium* from the homogenized root tissue sample in selective 1A broth (Brisbane and Kerr, 1983) followed by incubation at 26-28 °C for 48 hrs. Testing of DNA extracted by both methods, to detect rhizogenic *Rhizobium* spp., used the TaqMan® qPCR assay of Weller and Stead (2002) with primers rol-F/rol-R and probe rol-Pr. Results were compared from testing roots from tomato seedlings inoculated, as described above, with different concentrations of *Rhizobium* isolates.

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Objective 3 - To quantify the effect of biological-based products applied during propagation on infection and transformation of roots and incidence and severity of root mat disease

Preliminary inoculation trial – ADAS Boxworth

A trial was established in a glasshouse at ADAS Boxworth to examine the efficacy of different inoculation methods and timings, to ensure successful inoculation in the subsequent trial screening biological products. The trial also included both grafted (cv. Elegance grafted onto vigorous rootstock Emperador) and ungrafted (Elegance grown on its own roots) plants of a vegetative scion variety. The treatments tested can be seen in Table 2, below. Each treatment was replicated five times, and each plot (a single clean plastic tray) contained 4 plants.

Table 2. A summary of treatments applied to the preliminary inoculation trial – ADAS Boxworth, 2016

Trt	Inoculated	Timing	Rootstock	Isolate*	Wounding
1	-	-	Own-root	-	-
2	-	-	Emperador	-	-
3	✓	Immediately after transfer to cube	Own-root	Mix of 3	Plug edge damage
4	✓	Immediately after transfer to cube	Emperador	Mix of 3	Plug edge damage
5	✓	2 weeks after transfer to cube	Own-root	Mix of 3	Slice into cube
6	✓	2 weeks after transfer to cube	Emperador	Mix of 3	Slice into cube

Plants were sown into rockwool plugs on 7th April, and arrived on site on 25th April. Plants were transferred to rockwool propagation cubes on the day of arrival. All plants were treated with Nemasys (*Steinernema feltiae*) before entering the glasshouse, to reduce the risk of sciarid fly spreading root mat between treatments. Roots of the tomato seedlings had reached the edge of the plug, and were in contact with the polystyrene plug trays. For treatments 3 and 4, rough removal of plugs from the plug tray was considered a sufficient wounding treatment prior to inoculation. This slight wounding to young roots at transplanting may also emulate commercial situations.

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The inoculum was generated at Fera Science Ltd, Sand Hutton and transported to ADAS Boxworth on 26-04-2016. For the inoculation of treatments 3 and 4, inoculum was prepared in the form of 24 hour cultures of known rhizogenic strains of *R. radiobacter* on nutrient dextrose (ND). Plates were flooded with sterile distilled water (SDW) and agitated to generate a stock suspension of inoculum. This was then diluted to a strength of 1 x 10^8 cfu/ml with SDW. Three different rhizogenic isolates (NCPPB 4062, P6994 and Pr20E9) were combined to produce the final inoculum. Inoculum strength was confirmed by dilution plating on ND agar. Each plant to be inoculated at this time received a 20 ml drench of this inoculum, poured over the rockwool plug.

Treatments 5 and 6 were inoculated two weeks later on 10th May, using fresh inoculum sent via courier by Fera Science Ltd. The inoculum was prepared the same way as previously, using the same number of *R. radiobacter* agar plates of the same age as previously. Plants were wounded by making an approximately 8 cm long cut into the cubes with a new, clean scalpel. The bottom of cubes was checked to determine the distance into the cube roots had spread, to ensure wounding was effective. Plants were wounded on one side only, so that the area most likely to show early root mat symptoms was the same on each cube. Each plant to be inoculated at this time received a 20 ml drench of this inoculum, poured into the cut made on cubes. Around each inoculation event, irrigation was scaled back to create a drier environment around roots, as this has been shown to facilitate root infection.

Plants were fertilised and irrigated using a dosatron, programmed to irrigate all plots equally with 300 mls water and soluble feed each hour. EC and pH of the feed was checked periodically, and water content, temperature and EC of cubes was also monitored throughout the trial. A temperature and humidity logger was also placed in the glasshouse. The plants were grown under a very wet irrigation regime to encourage root mat symptom expression. Following inoculation plants were monitored for development of symptoms (as well as any other issues), and following the arrival of suspected root mat, the trial was assessed twice weekly. Plants in each plot were assessed for root mat incidence and severity of symptoms, expressed as % of the cube surface affected. Plants were grown on for approx. 14 weeks after arrival. At the end of the trial, plants were destructively assessed, where the propagation cube was split open and extent and severity of roots within and underneath the cube were also assessed. Crop vigour was assessed throughout the trial on a 0-5 scale, where 0=dead plant and 5=healthy, green and vigorous. When first symptoms were suspected, and again following the final assessment, root samples were sent to Fera for testing with the new diagnostic, detailed under Objective 2.

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Biological product screening trial – ADAS Boxworth

Following the preliminary trial, a number of plant protection products, biostimulants and plant strengtheners were evaluated for their effect on root mat infection and symptom expression. To facilitate a greater number of treatments and a greater number of plants per plot, this trial was established in a polytunnel at ADAS Boxworth in autumn 2016. Plants were sown on 21st July and arrived on site on 3rd August. Plants in this trial were all ungrafted and of cv. Elegance, and were directly sown into rockwool propagation cubes. All plants were treated with Nemasys to reduce the risk of sciarid fly spreading root mat between treatments.

On arrival, plants were wounded by making an approximately 8 cm long cut into the cubes with a new, clean scalpel. The bottom of cubes was checked to determine the distance into the cube roots had spread, to ensure wounding was effective. Plants were wounded on one side only, so that the area most likely to show early root mat symptoms was the same on each cube. One treatment (Treatment 3) remained unwounded. Following wounding, experimental treatments, as summarised in Table 3 below, were applied.

Table 3. A summary of the treatments, largely biological, applied to plots for control of root mat – ADAS Boxworth, 2016

Treatment	**Product**	**a.i.**
1	Untreated uninoculated	water
2	Untreated inoculated	water
3	Unwounded inoculated	water
4	Trianum P	*Trichoderma harzianum* T-22
5	ProParva	Plant auxins
6	Jet 5	Hydrogen peroxide
7	Proradix	*Pseudomonas* sp. DSMZ 13134
8	Serenade ASO	*Bacillus subtilis* QST 713
9	Carbon Gold Biology Blend	Enriched biochar, microbes, wormcasts, seaweed etc.
10	Trianum P Serenade ASO	Combination of these 2 treatments
11	Additional booster treatment of T10 24 hours after inoculation	Combination of these 2 treatments

Application rates and methodology was guided by manufacturer/distributor recommendations. Untreated plots or those where the product was not in liquid formulation received drenches of SDW in place of made-up liquid product. Treatments were applied immediately after wounding, as propagation cubes were transferred to new rockwool slabs

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(Grodan Vital). There were six plants in a plot, with three plants grown on each of two slabs. Slabs were placed on top of clean crates so that run-off was unable to spread between plots. For treatment 7, an experimental product, run-off was collected in trays placed below the crates over the course of the trial, and was then disposed of in an on-site Sentinel.

The trial was inoculated 24 hours after treatment, again using inoculum generated by Fera (see above). The plastic around the slabs was slit prior to inoculation, to provide a slightly drier root environment at inoculation to facilitate transformation of roots. Each plant to be inoculated received 20 ml of inoculum, poured into the cut made in cubes at wounding. Uninoculated plants received 20 ml of SDW.

Plants were irrigated throughout the trial with soluble feed using a dosatron following commercial practice. EC, pH, substrate temperature and water content were monitored throughout the trial. A temperature and humidity logger was also placed in the polytunnel. Plants were grown on for 14 weeks, and were pruned appropriately throughout the trial. By 20th October plant heads had reached the wire, and three trusses had set fruit, so the heads were taken out.

Following inoculation, plants were monitored for development of symptoms (as well as any other issues), and following the arrival of suspected root mat, the trial was assessed twice weekly. Plants in each plot were assessed for root mat incidence and severity of symptoms, expressed as % of the cube surface affected. At the end of the trial, plants were destructively assessed, where the propagation cube was split open and extent and severity of roots within and underneath the cube were also assessed. Crop vigour was assessed throughout the trial on a 0-5 scale, where 0=dead plant and 5=healthy, green and vigorous. When first symptoms were suspected, and again following the final assessment, root samples and samples of set fruit and their seed were sent to Fera for testing with the new diagnostic, detailed under Objective 2.

Data from both trials were analysed by Analysis of Variance.

Determining the pathogenicity of isolates – Fera

Pathogenicity of the three rhizogenic isolates used in this experiment was examined as described under Objective 2 (see Table 7 and Figure 6). All three isolates induced root mat symptoms within 5 weeks under experimental conditions in the glasshouse at Fera when inoculated onto damaged roots at concentrations above 10^6 cfu per ml.

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In late 2016, commercial sites were selected to be part of a trial testing products applied both during propagation in Holland (Objective 3) and during cropping (Objective 4). Plants will be tested for infection using the new diagnostic at plant arrival in 2017, and the development of root mat monitored and assessed over the course of the season in two large scale trials.

Additional observations

Monitoring of root mat incidence and severity in difference coir substrate mixes

In late 2015, it came to researcher's attention that a commercial site with three different brands of coir slab were observing severe root mat symptoms on site. The site was visited on 19th August, and root mat symptoms assessed in approximately 1000 plants per slab type. In 2016, this visit was repeated, on 7th October. Two blocks with Brand 3 slabs were assessed separately, as incidence of root mat reportedly differed between two scion varieties. The slab types involved are summarised in Table 4.

Table 4. A summary of coir slab types as used in a commercial tomato crop and their individual properties

Year	Coir brand	Chip/pith ratio	Chip size
2015	Brand 1	50:50	Standard
2015	Brand 2	40:60	Variable
2015	Brand 3	70:30	Standard
2016	Brand 1	60:40	Standard
2016	Brand 2	70:30	Variable
2016	Brand 3	70:30	Standard
2016	Brand 3	70:30	Standard

Plants were assessed for root mat incidence, and severity was assessed on a 0-5 scale (0 representing no root mat, 3 representing a swollen, badly affected cube, 5 representing a swollen, badly affected slab). Samples of the least and worst affected slabs were sent for tests to determine key properties, e.g. air filled porosity.

Commercial trial, USA

In 2016, a grower of cucumber and tomato in Texas, USA, with root mat problems in both crops, sought guidance for the set-up of a trial of the product Prestop (*Gliocladium catenulatum*) applied as a drench to the root zone to control the disease. Prestop was applied

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twice, at a rate higher than that recommended in the UK (approx. 15 ml per cube), once at wetting up cubes and again just before dispatch from the propagation nursery. In addition, the removal of plastic wrappers from cubes was examined alone or in combination with the Prestop treatment. A plan for a randomised block trial with four replicates of four treatments was designed and supplied by ADAS. This experiment was established in a 1 ha crop of cucumber cv. Verdon grown on Cultilene slabs with with 100 plants per plot assessed. Plants were monitored over the season for development of root mat. Root mat incidence and severity (on a 0-5 scale as detailed above) was assessed on two occasions. Data was then sent to ADAS UK Ltd for analysis and interpretation.

Results

Objective 1 - To review and summarise current knowledge of root mat disease in tomato and cucumber through production of text and photographs for an HDC Factsheet/review document.

Root mat in tomato is caused by rhizogenic plasmids (pRi), and crown gall is caused by tumorigenic plasmids (pTi), most commonly vectored by *Rhizobium radiobacter*, a common soilborne bacterium. Bacteria causing root mat and crown gall may both acquire and lose these plasmids. Bacteria may also carry a varying number of additional plasmids. The presence of the bacteria is required to transfer T-DNA from the plasmid to the plant genome. There is no evidence that Ri and Ti plasmids in the absence of bacteria will cause disease symptoms. Recently, the genus *Rhizobium* was revised to incorporate all species previously described as *Agrobacterium*. This classification was based on 16S ribosomal DNA analysis and hence genetic relatedness. The genus *Agrobacterium*, originally established to contain plant pathogenic species closely related to *Rhizobium*, was considered to be an artificial genus.

In the review we use the current accepted name *Rhizobium radiobacter*, with the qualifying descriptor ‘rhizogenic strain’, to identify the cause of tomato and cucumber root mat. Where we report on experimental work on different crop species, and crown gall rather than root mat, we have generally retained the original *Agrobacterium* species name as given in the particular reference in order to avoid introducing possible errors (there is not a unique one-to-one translation between *Agrobacterium* and *Rhizobium* species names) and unnecessary complexity.

The development of crown gall (and also likely root mat) is activated by fresh wounds on roots or stems which produce exudates that act as signal molecules; bacteria move to the wound site along the chemical gradient. Infection occurs when a piece of the plasmid DNA, known as the transferred DNA (T-DNA), is transferred from the bacterium and incorporated into the

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host plant nuclear DNA. Genes contained on the plasmid are expressed when inserted into the plant genome leading to a plant hormone imbalance that results in tumour growth (crown gall) or uncontrolled root proliferation (root mat) at the infection site. Infected plant cells synthesise simple novel metabolites, known as opines, that are not found in normal plant tissues. The pattern of opines synthesised is determined by the type of virulence plasmid in the bacterium and, in general, the virulence plasmids also confer on the infecting bacterium the ability to utilise the same opines as nutrients.

In inoculation experiments, both inoculum concentration and plant age have been found to influence infection success and severity of symptoms. Tomato root mat disease does not appear to spread rapidly between plants on production nurseries after planting out. It is quite common to find rockwool or coir slabs with one plant severely affected and other plants in the same slab displaying no symptoms. Possibly this is because plants become less susceptible to transformation by the Ri plasmid as they age. Substrate type has also been found to affect root mat and both incidence, and severity of symptoms has been observed to differ between different types of coir. There is relatively little information on the effect of imposed tissue wounding on susceptibility of plants to root mat. The literature on crown gall clearly demonstrates that tissue wounding is important for infection of plants by tumorigenic *R. radiobacter*, and although experiments to date have not demonstrated a requirement for imposed root damage to permit development of root mat in tomato or cucumber, it is possible that the experimental procedures used for growing the plants have produced sufficient root damage to allow infection, or natural wound sites around emerging lateral roots provide the infection court.

Once a plant is infected with the Ri or Ti plasmid, there are no known treatments which will prevent symptom development. Consequently, the current focus for control of both root mat and crown gall disease is to prevent infection. As with other plant diseases, this may be achieved by host resistance, by environment manipulation to make conditions unfavourable for infection, or by reduction/elimination of rhizogenic *R. radiobacter* inoculum in the environment around plants. Most of the tomato varieties and rootstocks currently grown in the UK appear to be susceptible to root mat. Cultivar resistance to crown gall has been reported (e.g. in rose) and one tomato variety (cv. Kanavaro) has been observed to be less susceptible to root mat than others. It is possible that increased cultivar resistance to root mat in tomato may be identified. No root zone environment manipulation treatments that reliably reduce root mat have yet been identified. There is speculation that oxygen level in irrigation solution and irrigation frequency may influence the disease. There is good reason to believe biological treatments could reduce tomato root mat by influencing the population of rhizogenic bacteria around tomato roots. Specifically, recent work on crown gall disease showed that a

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quorum sensing signal is produced by populations of *A. tumefaciens* that controls transfer of the Ti plasmid. Transfer of the Ti plasmid only occurs at high population densities of *A. tumefaciens*, when concentration of the signalling molecule is high. Various isolates of *Bacillus*, *Pseudomonas* and *Trichoderma* species have been shown to reduce crown gall, possibly through reduction of *A. tumefaciens* populations. Assuming quorum sensing also operates with root mat disease, biological products might reduce root mat if they prevent the population reaching a threshold concentration where plasmid transfer occurs. Modified strains of *Agrobacterium* have shown most promise in control of crown gall and some (e.g. Galltrol) are marketed for this purpose, although not in the UK; *Agrobacterium radiobacter* K84, the active ingredient of Galltrol is considered to be a genetically modified organism by regulatory authorities and currently this prevents registration in the UK. Previous trials with biological products for control of root mat were largely unsuccessful due to low incidence and/or high variation in disease occurrence. A number of products, the majority of which are biological, were tested in a primary screen at ADAS Boxworth in 2016.

Various knowledge gaps pertinent to the control of root mat were identified and are listed below as a series of questions.

Sources of infection

1. Does rhizogenic *R. radiobacter* occur on commercial batches of tomato seed?
2. Is rhizogenic *R. radiobacter* present in irrigation water or growing media on propagation nurseries? Or associated with sciarid flies or other insects that frequent the tomato root zone?
3. Can the Ri plasmid persist in the environment in the absence of *R. radiobacter* or other vectoring bacteria?
4. Is there latent root mat infection in tomato plants at receipt on production nurseries?

Control by host resistance

5. What is the relative susceptibility to infection of:
    - Seedlings germinating in plugs (propagation nursery)
    - Young plants growing in cubes (propagation nursery)
    - Young plants rooting into slabs (production nursery)
    - Plants well established on slabs (production nursery)?
6. Is there a useful level of resistance to root mat in any tomato genotypes?
7. Can induction of host resistance (Systemic Acquired Resistance or Induced Systemic Resistance) in tomato provide any control of root mat?

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Control by inoculum reduction

8. How effective are microorganisms, biological preparations and biocides at maintaining rhizogenic *R. radiobacter* at nil or low population levels in the root zone and the wider glasshouse environment?

9. Does hypochlorite treatment of tomato seed for *Pepino mosaic virus* adequately control any *R. radiobacter* on/in seed?

Control by environment manipulation

10. Can we reduce opine accumulation to deprive *R. radiobacter* of nutrition and prevent population increase?

11. Does handling of plug plants or propagation blocks result in root damage sufficient to significantly influence susceptibility to infection? If so, can handling practices be adapted to minimise root damage and reduce infection?

12. Can we mask/interfere with phenolic compounds produced by tissue wounds and thereby reduce movement of rhizogenic *R. radiobacter* towards susceptible root tissue?

13. Does hypochlorite treatment of tomato seed increase susceptibility to infection by rhizogenic *R. radiobacter* by removal of non-pathogen strains and/or other competing microorganisms?

14. Would application of non-pathogenic microorganisms to seeds soon after hypochlorite seed treatment, especially root colonising bacteria, reduce the susceptibility of young plants to root mat, for example by colonisation of natural wound sites where lateral roots emerge?

15. Does irrigation solution temperature, pH, oxygen level, conductivity, nutrient form or level significantly influence the susceptibility of tomato roots to infection by rhizogenic *R. radiobacter*?

16. Does the water holding capacity of a slab, profile of water distribution in a slab, or irrigation frequency, influence susceptibility of tomato plants to root mat?

17. Do environmental and crop management actions directed at switching plants from generative to vegetative growth increase susceptibility to root mat? Does induction of vegetative growth result in increased lateral root production?

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Objective 2 - To develop and fully validate a rapid molecular test for detection of T-DNA from different Ri plasmids in tomato roots prior to symptom occurrence

Rhizobium isolates

All selected isolates indicated as rhizogenic on tomato or cucumber tested positive for the cucumopine Ri plasmid according to both the Haas PCR and the qPCR of Weller and Stead and were found within *Rhizobium radiobacter* biovar 1 (Table 5).

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Table 5. UK *Rhizobium* isolates from tomato and cucumber plants with root mat symptoms

Isolate	Organism name	Year	Country	Host	Biovar	Haas PCR	Weller & Stead qPCR
NCPPB 2655	*Rhizobium radiobacter*	1974	UK	*Cucumis sativus*	1	+	+
NCPPB 2656	*Rhizobium radiobacter*	1974	UK	*Cucumis sativus*	1	+	+
NCPPB 2657	*Rhizobium radiobacter*	1974	UK	*Cucumis sativus*	1	+	+
NCPPB 2659	*Rhizobium radiobacter*	1974	UK	*Cucumis sativus*	1	+	+
NCPPB 2660	*Rhizobium radiobacter*	1974	UK	*Cucumis sativus*	1	+	+
NCPPB 3891	*Rhizobium radiobacter*	1994	UK	*Cucumis sativus*	1	-	-
NCPPB 4327	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
NCPPB 4328	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
NCPPB 4334	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
P 2527	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
P 2535	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
P 2546	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
P 2561	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
P 2581	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
P 2589	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	-	-
P 2609	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
P 2626	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	-	-
P 2631	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
P 2645	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	-	-
P 2658	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
P 2662	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
P 2666	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
P 2758	*Rhizobium radiobacter*	1997	UK	*Cucumis sativus*	1	+	+
NCPPB 4042	*Rhizobium radiobacter*	1998	UK	*Cucumis sativus*	1	-	-
NCPPB 4043	*Rhizobium radiobacter*	1998	UK	*Cucumis sativus*	1	+	+
P 3048	*Rhizobium radiobacter*	1998	UK	*Cucumis sativus*	1	+	+
P 3089	*Rhizobium radiobacter*	1998	UK	*Cucumis sativus*	1	+	+
P 3098	*Rhizobium radiobacter*	1998	UK	*Cucumis sativus*	1	+	+
P 3105	*Rhizobium radiobacter*	1998	UK	*Cucumis sativus*	1	+	+
P 3136	*Rhizobium radiobacter*	1998	UK	*Solanum lycopersicum* cv. Espero	1	+	+
P 3139	*Rhizobium radiobacter*	1998	UK	*Solanum lycopersicum* cv. Espero	1	+	+
P 3263	*Rhizobium radiobacter*	1998	UK	*Solanum lycopersicum* cv Conchita	1	-	-
P 3272	*Rhizobium radiobacter*	1998	UK	*Solanum lycopersicum* cv Favorita	1	+	+
NCPPB 4060	*Rhizobium radiobacter*	1999	UK	*Solanum lycopersicum* cv. Favorita	1	-	-
NCPPB 4062	*Rhizobium radiobacter*	1999	UK	*Solanum lycopersicum* cv. Espero	1	+	+
P 3576	*Rhizobium radiobacter*	1999	UK (Lancs)	*Solanum lycopersicum* cv. Golden Cherry	1	+	+
P 3577	*Rhizobium radiobacter*	1999	UK (Lancs)	*Solanum lycopersicum* cv. Golden Cherry	1	-	-
P 3815	*Rhizobium radiobacter*	2000	UK (East Yorks)	*Solanum lycopersicum*	1	+	+
P 3818	*Rhizobium radiobacter*	2000	UK (East Yorks)	*Solanum lycopersicum* cv. Cadence	1	+	+
P 3933	*Rhizobium radiobacter*	2000	UK (I of W)	*Solanum lycopersicum*	1	-	-
P 5088	*Rhizobium radiobacter*	2003	UK	*Solanum lycopersicum*	1	+	+
P 5120	*Rhizobium radiobacter*	2003	UK (I of W)	*Solanum lycopersicum*	1	+	+
P 5130	*Rhizobium radiobacter*	2003	UK (I of W)	*Solanum lycopersicum*	1	+	+
P 5136	*Rhizobium radiobacter*	2003	UK (I of W)	Solanum lycopersicum	1	-	-
P 6392	*Rhizobium radiobacter*	2006	UK	*Solanum lycopersicum* cv. Elegance	1	+	+
P 6399	*Rhizobium radiobacter*	2006	UK	*Solanum lycopersicum* cv. Claree	1	+	+
P 6998	*Rhizobium radiobacter*	2007	UK	*Solanum lycopersicum* cv. Elegance	1	+	+
TRK1	*Rhizobium radiobacter*	2015	UK (I of W)	*Solanum lycopersicum* cv. Arlinta	1	+	+
TRS1	*Rhizobium radiobacter*	2015	UK (I of W)	*Solanum lycopersicum* cv. Arlinta	1	+	+
TSS1	*Rhizobium radiobacter*	2015	UK (I of W)	*Solanum lycopersicum* cv. Arlinta	1	+	+
TSS2	*Rhizobium radiobacter*	2015	UK (I of W)	*Solanum lycopersicum* cv. Arlinta	1	+	+
P 2555	*Rhizobium* sp.	1997	UK	*Cucumis sativus*		-	-
P 2615	*Rhizobium* sp.	1997	UK	*Cucumis sativus*		-	-
P 3509	*Rhizobium* sp.	1999	UK (I of W)	*Solanum lycopersicum*		-	-
P 3512	*Rhizobium* sp.	1999	UK (I of W)	*Solanum lycopersicum*		-	-

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Ten *R. radiobacter* isolates from tomato or cucumber tested negative with both PCR and qPCR tests, either because they have since lost the plasmid or were non-rhizogenic at the time of isolation. Tomato and cucumber isolates not recognised as *R. radiobacter* also tested negative in both tests (Table 5).

The *R. radiobacter* reference strain P 5659 (Table 6) isolated from melon in Japan, which carries a mikimopine Ri plasmid containing the target sequence of the Haas PCR but not the target sequence of the Weller & Stead qPCR. All other rhizogenic reference strains of different *Rhizobium* spp., isolated from hosts other than tomato and cucumber (dahlia, sugar beet, rose, apple, daphne, prunus and grapevine) and known to carry other non-cucumopine Ri plasmids, all tested negative with both PCR and qPCR assays (Table 6).

Table 6. Other *Rhizobium* reference strains used in this study

Isolate	Organism name	Year	Country	Host	Biovar	Haas PCR	Weller & Stead qPCR
NCPPB 396	*Rhizobium radiobacter*	1957		*Dahlia* sp.	1	-	-
NCPPB 398	*Rhizobium radiobacter*	1957			1	-	-
NCPPB 1674	*Rhizobium radiobacter*	1963	UK	*Beta vulgaris*	1	-	-
P 5659	*Rhizobium radiobacter*	2004	Japan	*Cucumis melo*	1	+	-
NCPPB 2604	*Rhizobium* sp.	1974	Netherlands	*Daphne mezereum*		-	-
NCPPB 1855	*Rhizobium rhizogenes*	1959	USA	*Rosa* sp.	2	-	-
NCPPB 2270	*Rhizobium rhizogenes*	1969	UK	*Prunus avium*	2	-	-
NCPPB 2628	*Rhizobium rhizogenes*	1959	USA	*Rosa* sp.	2	-	-
NCPPB 2629	*Rhizobium rhizogenes*	1974			2	-	-
NCPPB 2991	*Rhizobium rhizogenes*	1977		*Malus*	2	-	-
NCPPB 3068	*Rhizobium rhizogenes*	1978				-	-
NCPPB 3269	*Rhizobium vitis*	1982	Afghanistan	*Vitis vinifera*	3	-	-
NCPPB 3270	*Rhizobium vitis*	1982	Afghanistan	*Vitis vinifera*	3	-	-

Pathogenicity tests

All isolates which tested positive for the cucumopine plasmid were rhizogenic on tomato seedlings (cv. Moneymaker), exhibiting clear root mat symptoms within 5 weeks after inoculation (Table 7). One atypical isolate of *R. radiobacter* (P3139) tested positive for the cucumopine Ri plasmid but did not cause root mat symptoms, presumably due to failure to infect through the wounded tomato roots. Isolates which tested negative for the cucumopine plasmid were non-pathogenic on tomato seedlings.

T-DNA rol-α target was successfully detected using the Weller and Stead qPCR assay in root samples taken from plants which developed root mat symptoms during pathogenicity testing. Rhizogenic bacteria were detected post-enrichment (C_T = <40.0) in roots from all symptomatic plants when tested after 5 weeks by the qPCR of Weller and Stead (Table 7). However, positive results were also obtained from non-symptomatic control plants inoculated with water only and from plants inoculated with non-rhizogenic strains (e.g. P3512). This was probably

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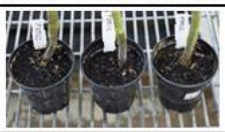
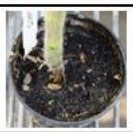
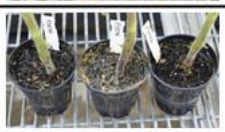
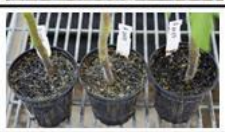
due to the high level of inoculum used (10^8 cfu per ml) and further multiplication of rhizogenic isolates and their spread in water splash during irrigation. Tomato roots sampled from the same batch of seedlings growing in another glasshouse, where rhizogenic *Rhizobium radiobacter* had not been introduced, tested negative (C_T = 40.0).

Table 7. Development of root mat symptoms 5 weeks after inoculation of roots with rhizogenic and non-pathogenic tomato and cucumber isolates of *Rhizobium radiobacter*. Lower CT values indicate higher population densities

Strain #	Symptoms (3 reps)	Close-up	Haas VirD2	Weller Taq-Man rol	Pathogenic-ity	Root wt. (g)	qPCR C_T
Control (H_2O)			-	-	-	29	22.4
P3512			-	-	-	33	34.3
P3139			+	+	-	27	19.9
N4062			+	+	+	94	20.3
P3576			+	+	+	87	21.0
P3136			+	+	+	82	20.2
Pr20E9			+	+	+	101	23.9
P6994			+	+	+	56	23.0
P6399			+	+	+	84	22.7
P3135			+	+	+	29	19.5

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Genome sequencing

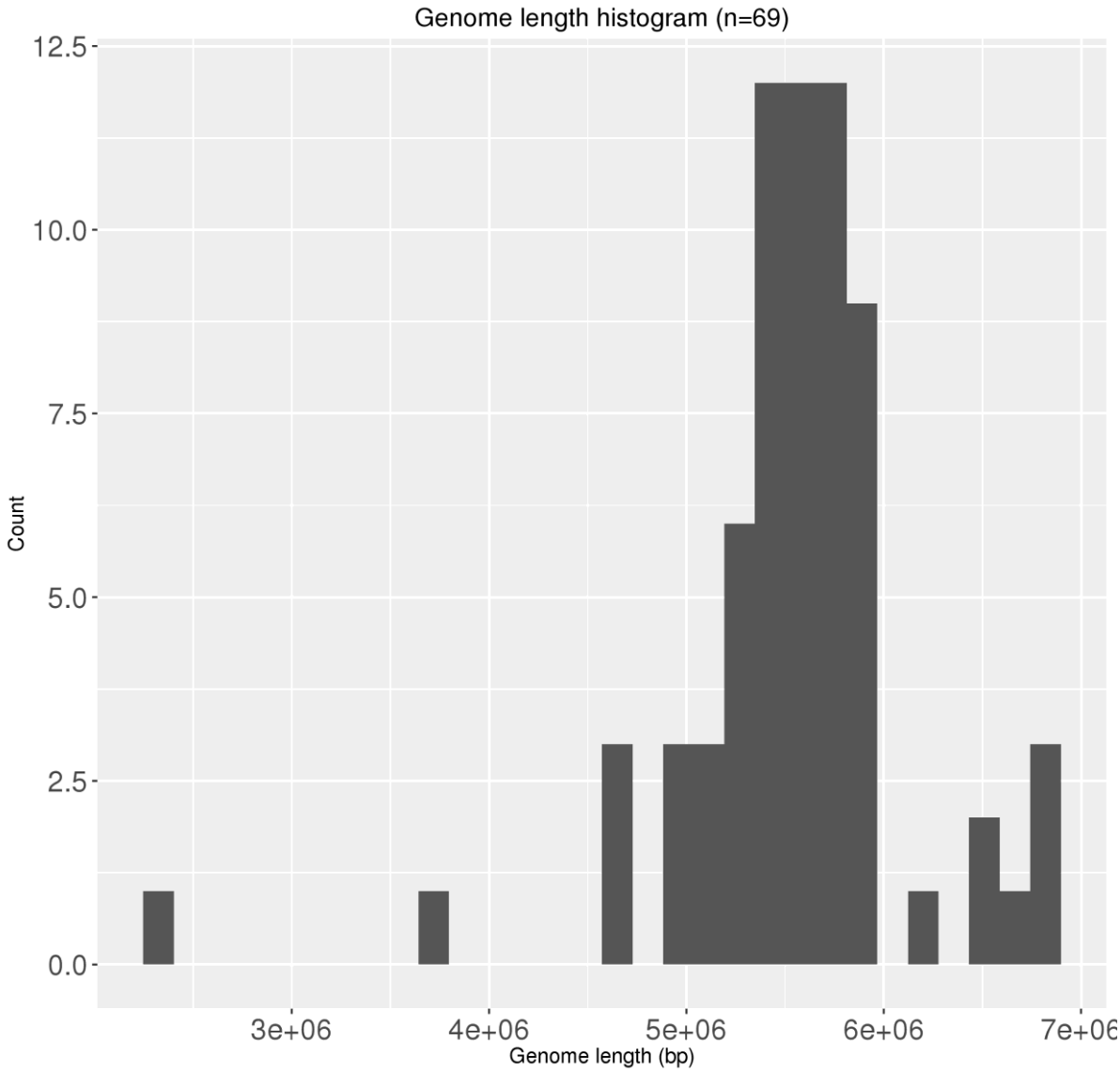

Figure 4. Estimated genome size of 68 sequenced *Rhizobium* isolates

The predicted genome sizes of all sequenced isolates ranged from 2.3Mbp to 6.8Mbp, with an average of 5.5Mbp (Figure 4). The presence of VirD2 and rol-α sequences, the T-DNA gene targets for Haas PCR and Weller and Stead qPCR were confirmed by blastn search in the genome of each rhizogenic tomato and cucumber isolate carrying the cucumopine Ri plasmid. Similarly, absence of these target sequences was confirmed in non-rhizogenic tomato and cucumber isolates and rhizogenic strains of *Rhizobium* spp. carrying other Ri plasmids.

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Average nucleotide identity comparison of all the sequenced strains was carried out with the software tool PyANI (https://github.com/widdowquinn/pyani) and were calculated with the ANIm metric (Figure 5). *R. radiobacter* biovar 1 isolates were highly heterogenous, clustering in 2 main phylogroups both with sub-clusters, each representing both rhizogenic and non-pathogenic tomato and cucumber isolates. A third phylogroup comprised 2 sub-clusters; one including isolates of *Rhizobium* biovar 2 (*R. vitis*) and non-pathogenic UK isolates of *Rhizobium* sp. from cucumber and the other including isolates of *Rhizobium* biovar 3 (*R. rhizogenes*) and non-pathogenic UK isolates of *Rhizobium* sp. from tomato.

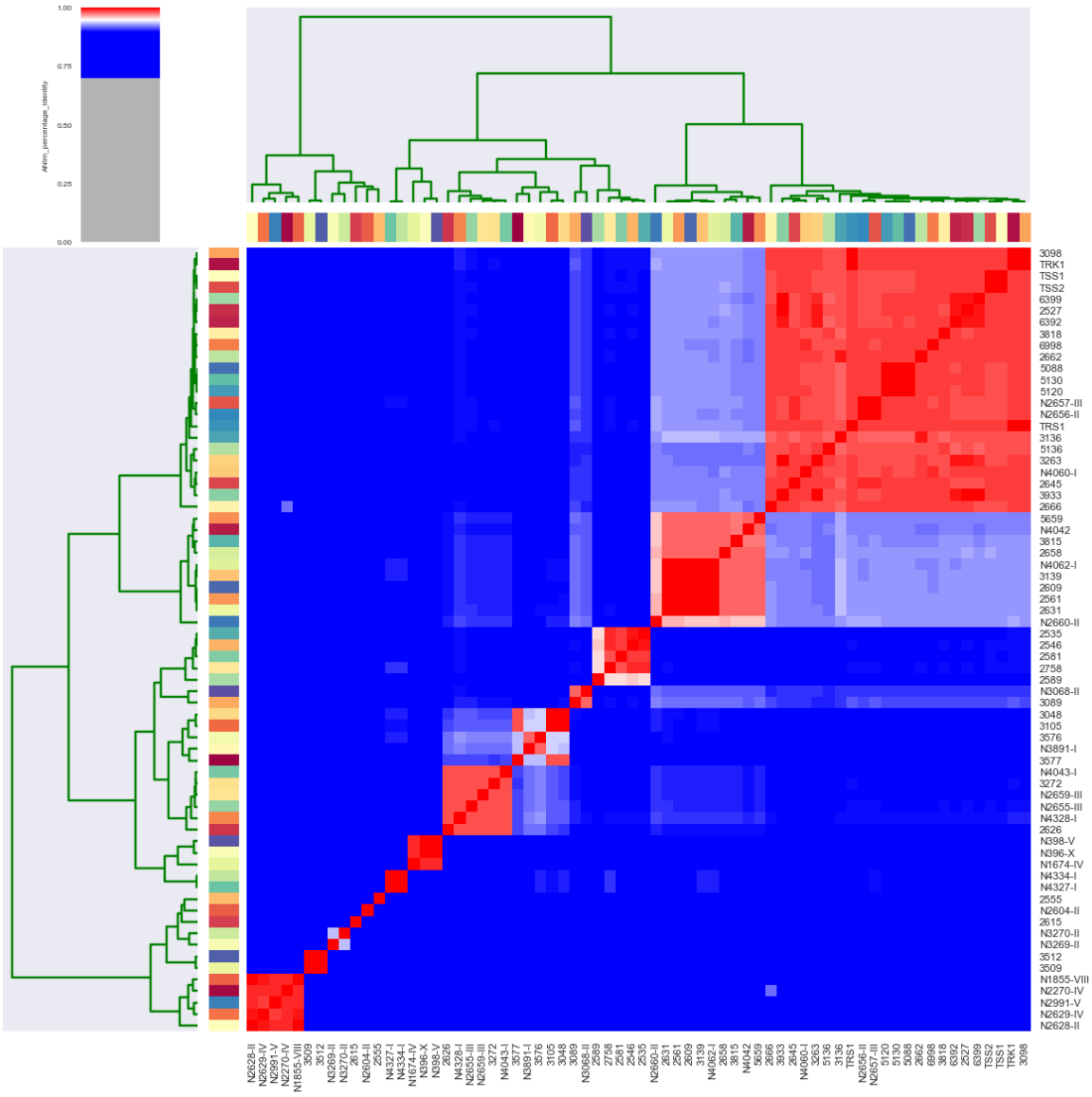

Figure 5. Average nucleotide identity genome comparisons of 68 *Rhizobium* isolates. Each square in the grid is a pairwise comparison between those two genomes and the colour of the grid shows the percent identity calculated between them. Red shows very closely related while blue is less closely related.

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Detection of T-DNA in infected tomato plants prior to development of root mat symptoms

To obtain tomato seedlings at different stages of symptom development, roots were inoculated as described above but with serially-diluted suspensions of selected rhizogenic isolates of *R. radiobacter* (Figure 6). When roots were sampled from plants inoculated with the lowest concentrations of bacteria (10^5 cfu per ml), positive qPCR results were obtained using either the established method involving pre-enrichment of bacteria in Schroth's selective medium (Weller and Stead, 2002) or after direct DNA extraction from the roots of infected plants using the newly developed test procedure (Figure 7). As previously, there was some evidence that control plants held in the same glasshouse became infected due to spread of rhizogenic bacteria from neighbouring inoculated plants. Healthy control plants from the same batch of tomato seedlings grown in a separate greenhouse tested negative.

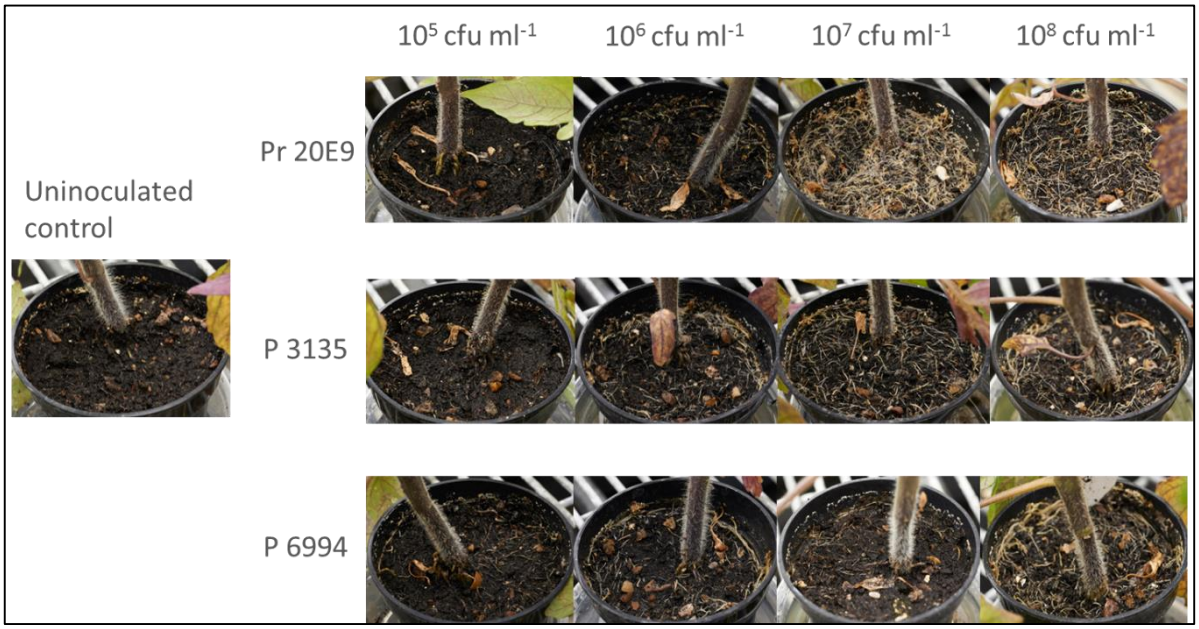

Figure 6. Infectivity titration with rhizogenic isolates of *R. radiobacter* showing different stages of symptom development after 5 weeks at 25 °C.

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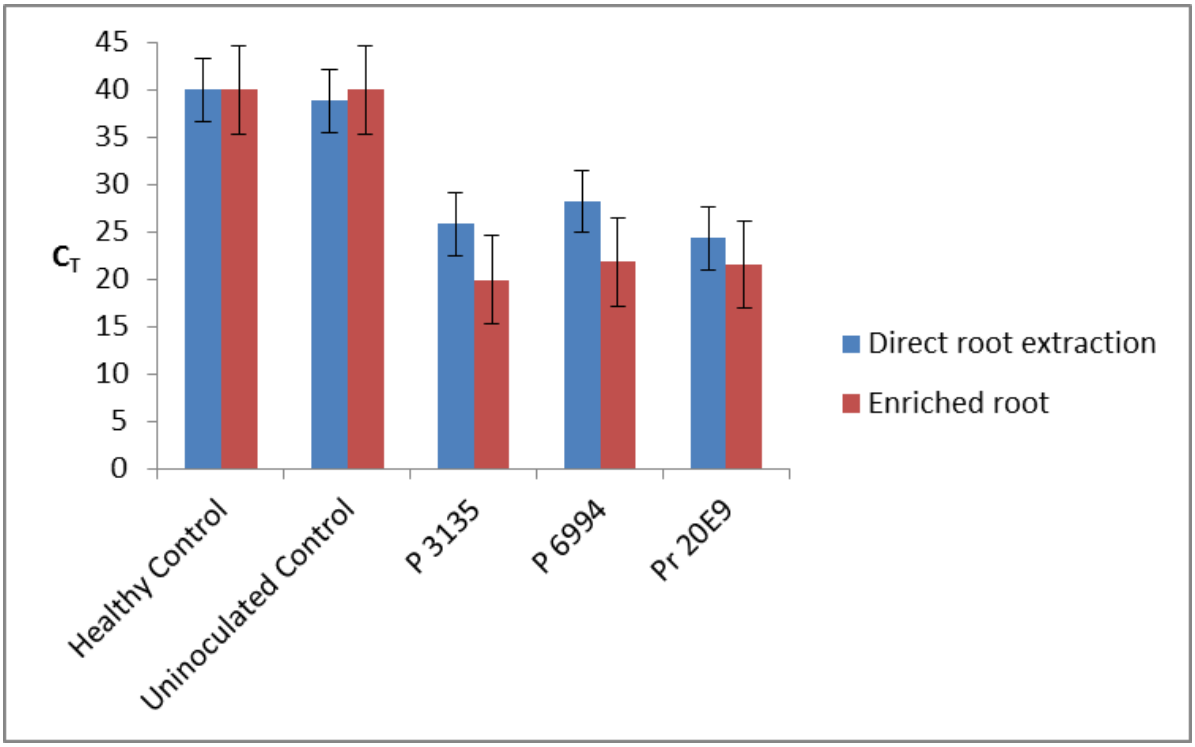

Figure 7. Mean C_T and standard error following testing of root samples from asymptomatic tomato seedlings by qPCR (Weller and Stead, 2002) following either enrichment in selective broth or direct DNA extraction and purification from root tissue.

Objective 3 - To quantify the effect of biological-based products applied during propagation on infection and transformation of roots and incidence and severity of root mat disease

Preliminary inoculation trial – ADAS Boxworth

Significant differences in crop vigour were recorded (p=0.002) at the initial assessment on 25 April, but this was related to whether plants were grafted or ungrafted, and was unrelated to root mat incidence. A view of trial set-up can is shown in Figure 8.

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Figure 8. Trial view showing set-up of propagation cubes in open trays - ADAS Boxworth, 2016.

First appearance of suspected root mat symptoms were noted on 17 May 2016, 21 days after the early inoculation. Upwards facing white roots were noted in the surfaces of some cubes, and were recorded as possible root mat (Figure 9). Plants inoculated 2 weeks later with more severe wounding also began to exhibit symptoms at a similar time (see Figures 11 and 12) and it is possible that this severe wounding treatment accelerated symptom progression.

Figure 9. An example of early root mat symptoms, originally noticed as suspect and then confirmed by molecular testing at Fera, and subsequent development of severe symptoms (visible dark growth on the cube is algae due to damp conditions) - ADAS Boxworth, 2016

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Root samples were taken from each treatment on 7 June and were sent to Fera for testing with the qPCR test of Weller and Stead (2002) (tested 14 June). The results from these tests are summarised in Table 8, and show Ct values indicating positive detection of the plasmid T-DNA in roots sampled from inoculated plots. The test also revealed low level infection in roots of uninoculated grafted plants and subsequently symptoms were observed in both this treatment and the own-root uninoculated plants.

Table 8. Results of Taqman PCR test for presence of T-DNA in tomato roots – Fera Science Ltd, June 2016

				qPCR result	
Trt	Inoculated	Timing	Rootstock	Ct value 1	Ct value 2
1	-	-	Own-root	40.00	40.00
2	-	-	Emperador	39.52	38.57
3	✓	Immediately after transfer to cube	Own-root	24.83	24.63
4	✓	Immediately after transfer to cube	Emperador	32.21	23.24
5	✓	2 weeks after transfer	Own-root	28.84	29.19
6	✓	2 weeks after transfer	Emperador	22.03	22.39

Symptoms remained at a low level for approximately 3 weeks, before increasing suddenly in severity, recorded at the 8 June assessment. At the final assessment on 29 June, symptoms were extremely severe in both grafted and ungrafted plants. Symptomatic grafted plant roots looked similar to the 'bale of hay' type roots observed in commercial crops, whereas symptomatic ungrafted plants exhibited long, straight and very white roots growing prolifically from the stem base (Figure 10).

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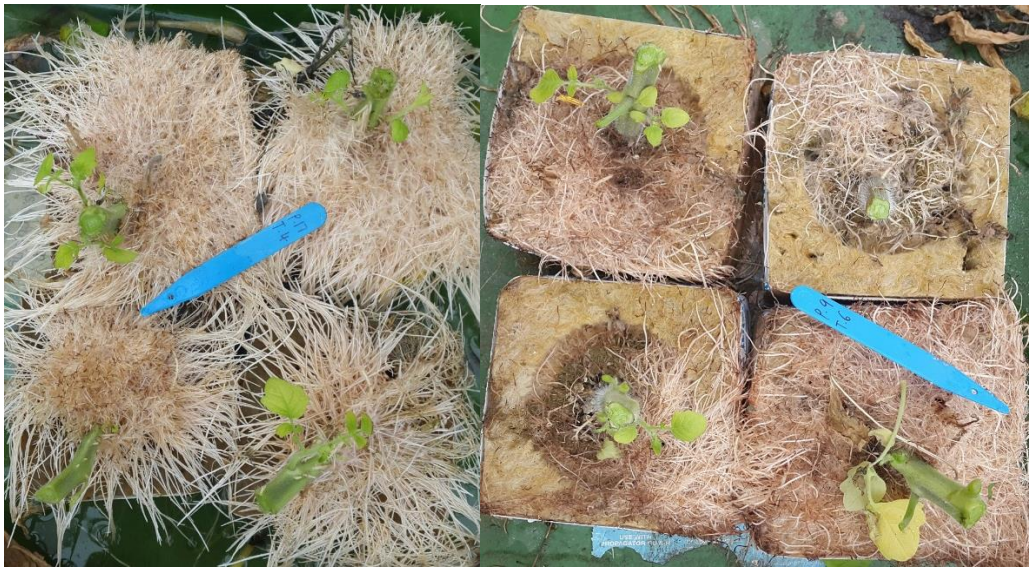

Figure 10. Representative examples of severe symptoms observed in ungrafted (left) and grafted (right) plants, 7-9 weeks after inoculation - ADAS Boxworth, 2016

The increase in incidence and severity following the appearance of first symptoms is summarised in Figures 11 and 12 below. As these figures illustrate, uninoculated plots also became infected over the course of the trial, and expressed symptoms comparable to inoculated plots by the trials conclusion. Water content measurements and temperature and humidity data can be seen in the Appendix. In terms of glasshouse conditions, symptoms did appear to increase rapidly when % water content of propagation cubes increased and following a prolonged period of high temperatures and more stable relative humidity. Uninoculated plots likely became infected via contamination from other plots. As trays were placed very close to each other, and often contained standing water, water splash is the most likely explanation for this. It is possible that as conditions that appear to have been favourable to *R. radiobacter* occurred in early June, a rapid population increase and a subsequent increase in symptom expression occurred in all infected plots, masking differences in initial inoculum concentration. The rapid increase in severity happened approx. 7 weeks after the first inoculation, which seems to agree with timescales observed commercially.

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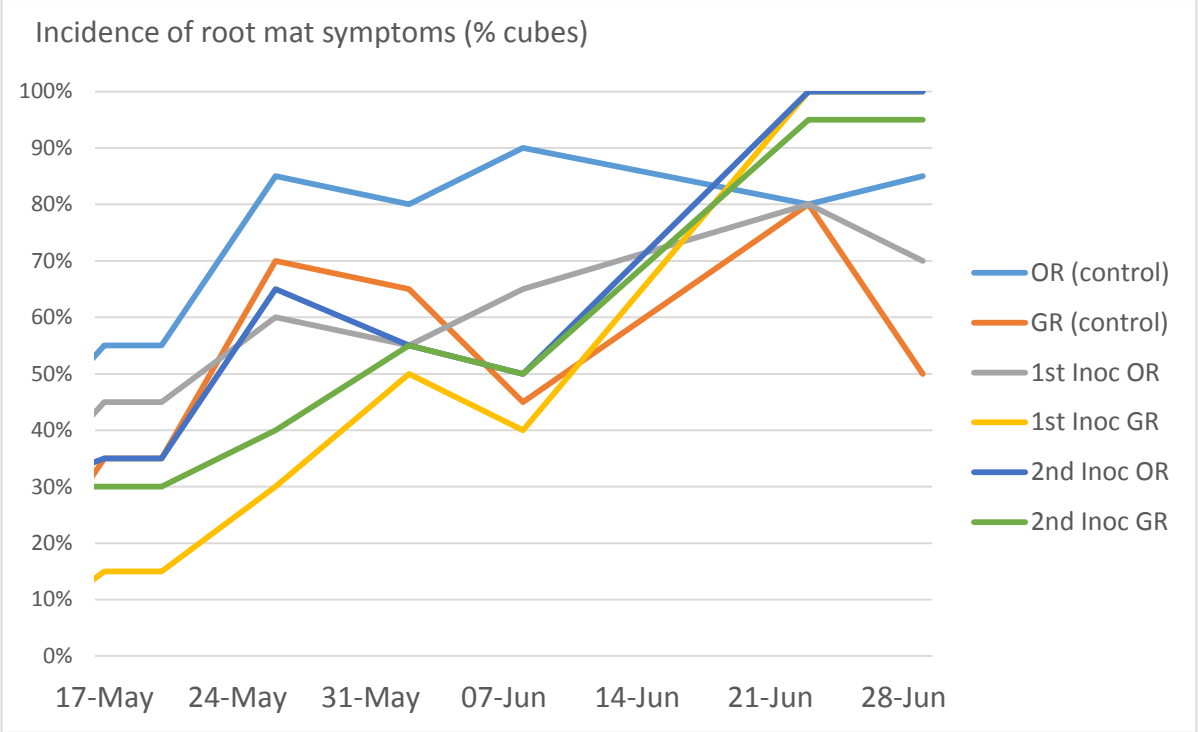

Figure 11. Incidence (% cubes affected) of observable root mat symptoms in inoculated and uninoculated cubes - ADAS Boxworth, 2016

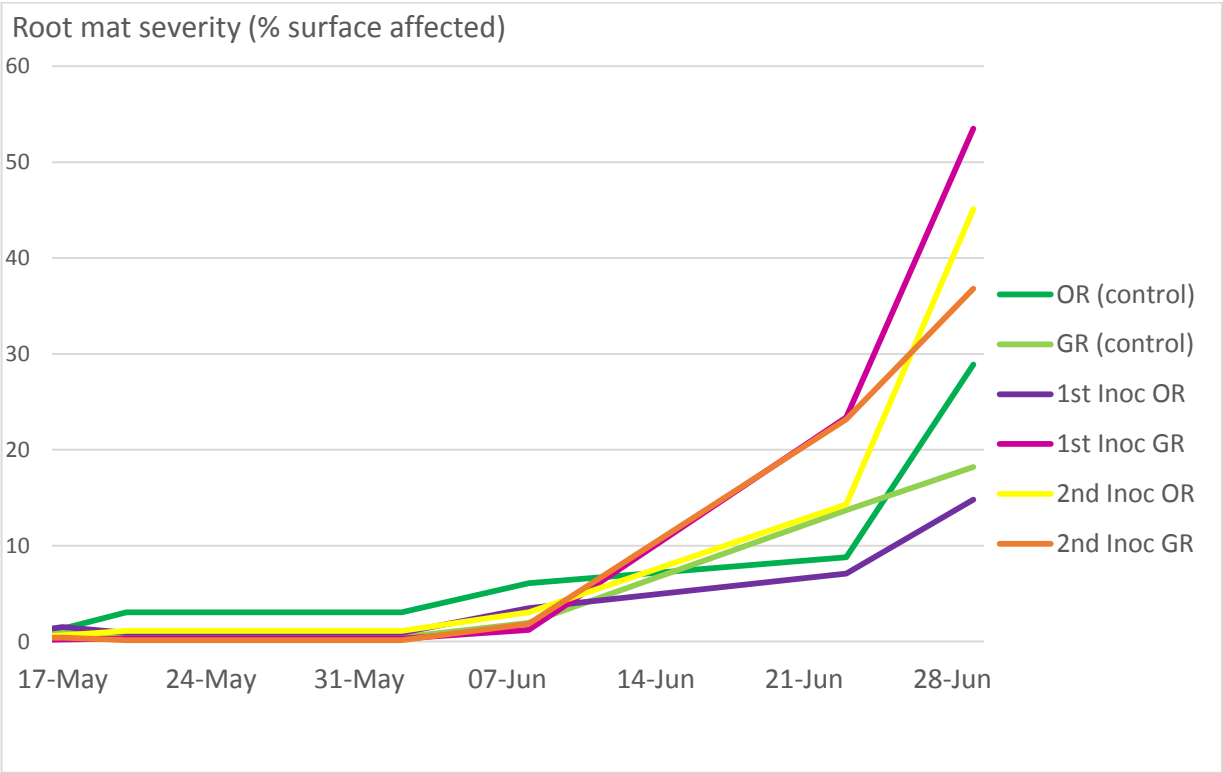

Figure 12. Severity (% cube surface with symptoms) of observable root mat symptoms in inoculated and uninoculated cubes - ADAS Boxworth, 2016

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Statistically significant differences in incidence were only recorded at the final assessment (63 days after first inoculation). It is, however, important to note that considering the TaqMan assay, incidence may have been nearly universal but with very low symptom expression recorded as nil. Statistically significant differences in severity were not present at the final assessment, but were recorded at assessments on May 26, June 2 (both with p=0.007) and 8 June (p=0.022). Here, recorded severities in uninoculated plots were higher than those in inoculated plots which makes comparison difficult.

Biological product screening trial – ADAS Boxworth

No issues were encountered in the mixing or application of any of the products included in this trial. Generally lower levels of symptom expression were achieved in this trial, which may be due to the generally drier growing environment as plants were grown on slabs as commercially rather than in open, wet trays (Figure 13).

Figure 13. Trial view showing five replicate blocks of tomato plants, approx. 12 weeks old - ADAS Boxworth, 2016.

Symptoms were first recorded on 26th August 2016, 22 days after inoculation. Symptoms here remained at the stage where white roots were visible on the surface of the cube, rather than to the more extreme symptoms observed previously. On destructive assessment of cubes and slabs, more extensive symptoms of root mat were noted. This may be as symptom

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expression was favoured more towards the bottom of the cube and slab, where conditions were damper. Most affected plots showed typical root growth around and into the irrigation peg, as is commonly observed on commercial sites. Symptom expression was also more obvious towards the side of the cube that received the *R. radiobacter* inoculum. Examples of the symptoms assessed can be seen in Figure 14.

Figure 14. Symptoms of root mat observed and recorded at final assessment; A - root matting at slab edges and corners; B - root mat present in cube showing root growth up the irrigation peg channel; C - characteristic long, white roots on infected slab; D - Root mat on propagation cube on the inoculated side and not the uninoculated side. - ADAS Boxworth, November 2016.

Incidence of symptoms was initially low, but climbed to near 100% of cubes by the end of August. By the end of the trial, almost all cubes were affected to differing degrees. There were no statistically significant differences between treatments in terms of cubes affected, though Treatment 7, Proradix, did result in significantly fewer cubes expressing symptoms when compared to inoculated cubes and some of the other treatments in the trial. Differences in % of the cube surface with root mat symptoms (severity) was not significantly different between symptoms over the course of the trial. Severity of root mat was low at the start of the trial, and did not take off until mid-October, possibly following an increase in irrigation to

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all plants in the trial. Incidence and severity data that could be observed over the course of the trial is summarised in Figures 15 and 16, and in Tables 9 and 10.

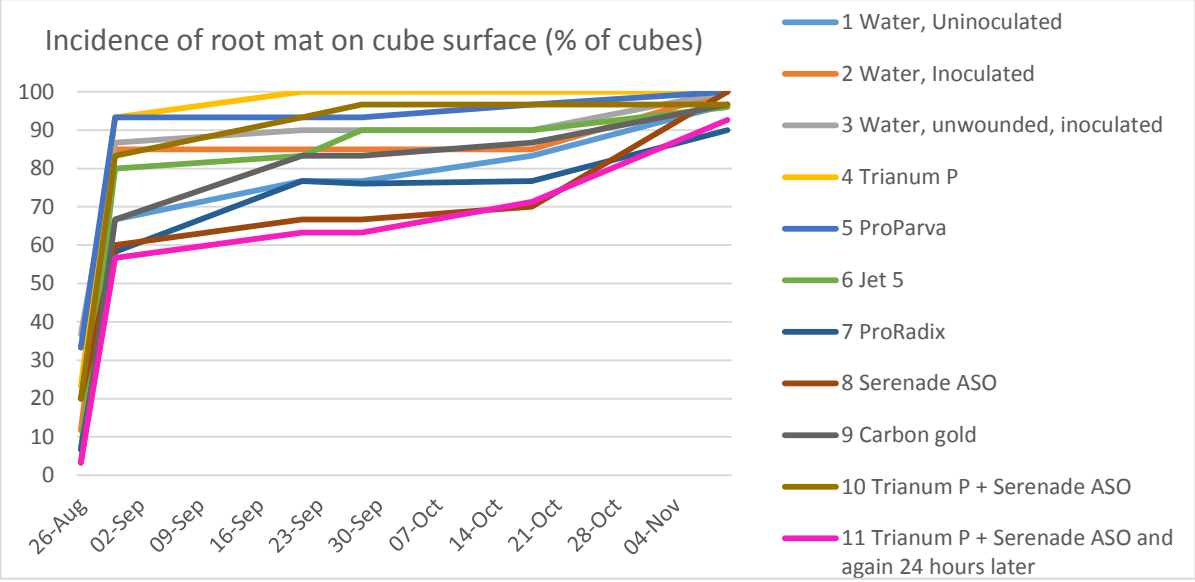

Figure 15. Incidence of root mat symptoms recorded over the course of product screening trial - ADAS Boxworth, 2016

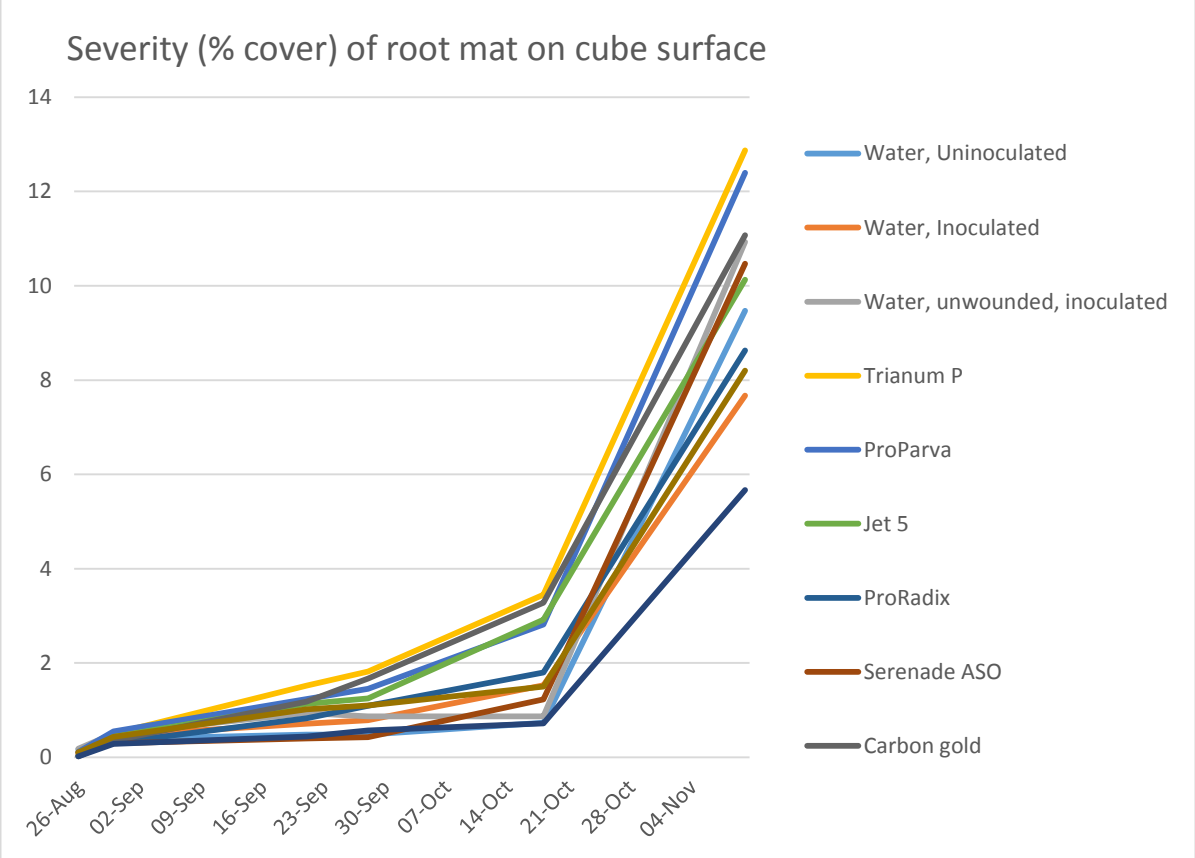

Figure 16. Severity of root mat symptoms recorded over the course of a product screening trial - ADAS Boxworth, 2016

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Table 9. Incidence of root mat symptoms assessed from first appearance to the trials final assessment - ADAS Boxworth, 2016

Treatment		Incidence of root mat symptoms (no. cubes affected)					
		26-Aug	30-Aug	21-Sep	28-Sep	18-Oct	10-Nov
1	Water, Uninoculated	33.3	66.7	76.7	76.7	83.3	96.7
2	Water, Inoculated	11.7	85.0	85.0	85.0	85.0	100.0
3	Water, unwounded, inoculated	36.7	86.7	90.0	90.0	90.0	100.0
4	Trianum P	23.3	93.3	100.0	100.0	100.0	100.0
5	ProParva	33.3	93.3	93.3	93.3	96.7	100.0
6	Jet 5	6.7	80.0	83.3	90.0	90.0	96.0
7	Proradix	6.7	58.3	76.7	76.07	76.7	90.0
8	Serenade ASO	20	60.0	66.7	66.7	70.0	100.0
9	Carbon Gold	3.3	66.7	83.3	83.3	86.7	96.7
10	Trianum P + Serenade ASO	20	83.3	93.3	96.7	96.7	96.7
11	Trianum P + Serenade ASO and again 24 hours later	3.3	56.7	63.3	63.3	71.3	92.7
p value		0.135	0.127	0.252	0.177	0.224	0.288
LSD		28.22	31.27	28.22	27.77	24.6	8.608

Table 10. Severity of root mat symptoms assessed from first appearance to the trial's final assessment - ADAS Boxworth, 2016

Treatment		% cube surface affected by symptoms of root mat					
		26-Aug	30-Aug	21-Sep	28-Sep	18-Oct	10-Nov
1	Water, Uninoculated	0.17	0.38	0.48	0.48	0.73	9.47
2	Water, Inoculated	0.06	0.46	0.71	0.79	1.53	7.67
3	Water, unwounded, inoculated	0.18	0.50	0.93	0.87	0.87	10.93
4	Trianum P	0.12	0.50	1.52	1.82	3.45	12.87
5	ProParva	0.12	0.55	1.23	1.45	2.82	12.40
6	Jet 5	0.03	0.43	1.13	1.25	2.92	10.13
7	ProRadix	0.03	0.31	0.83	1.09	1.80	8.63
8	Serenade ASO	0.10	0.30	0.40	0.43	1.23	10.47
9	Carbon Gold	0.02	0.35	1.18	1.67	3.28	11.07
10	Trianum P + Serenade ASO	0.10	0.43	1.02	1.10	1.50	8.20
11	Trianum P + Serenade ASO and again 24 hours later	0.02	0.28	0.44	0.57	0.72	5.67
p value		0.135	0.122	0.215	0.12	0.115	0.112
LSD		0.1411	0.2006	0.876	1.035	2.274	4.652

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To definitively confirm infection, roots from the top of cubes were sampled on 28th August and sent to Fera for molecular testing. On this occasion, both the new qPCR assay and an established root mat diagnostic involving an enrichment step were carried out for comparison (Table 11). The tests largely agree with one another, showing that all the roots sampled from the trial contained T-DNA to differing degrees (the lower the C_T score, the greater the amount of DNA detected). The more established test involving the enrichment step returns lower numbers as *R. radiobacter* is bulked up before testing by PCR, meaning more DNA is present in each sample, whereas the newer assay is carried out on extractions made from the roots themselves. The results of both tests show that the most T-DNA is present in Carbon Gold treated roots. It should be noted that the roots sampled were symptomatic and on top of the cube, and these results largely agree with assessments of % of the cube surface affected (Table 10 and Figure 16). In some cases, the test results do not agree, for example the direct extraction method shows a greater amount of T-DNA in the unwounded inoculated than in the wounded inoculated, whereas the enrichment method shows the opposite. It is as yet unclear how much individual C_T values can be interpreted, especially for the enriched samples where live cells will grow in the enrichment medium but dead cells will be diluted. As such it is recommended enrichment test results are taken as positive (C_T<40) or negative (C_T=40). In general, there is a 10-fold difference in target copies for every three CT values.

For the direct extraction, there should be a general link between CT and numbers of target sequences present in the sample extract but this will not discriminate between target in bacteria (live or dead) and target in transformed root cells. There may also be plant to plant variation depending on where the sample is taken.

Table 11. A comparison of two different molecular diagnostics used to detect root mat disease, confirming infection in all treatments (two replicate tests) - 2016

Trt no.	Treatment	Ri plasmid C_T			
		Direct root extraction		Enrichment	
1	Water, Uninoculated	32.27	32.38	25.39	25.52
2	Water, Inoculated	31.14	31.20	31.41	31.60
3	Water, unwounded, inoculated	26.08	25.99	35.07	35.01
4	Trianum P	33.01	32.33	26.71	26.74
5	ProParva	34.16	34.22	22.36	22.43
6	Jet 5	34.84	34.34	25.98	26.08
7	ProRadix	34.42	35.23	25.41	25.10
8	Serenade ASO	35.97	35.74	28.56	28.68
9	Carbon Gold	27.75	27.87	23.95	24.03
10	Trianum P + Serenade ASO	31.63	31.91	26.08	26.11
11	Trianum P + Serenade ASO + after 24 hrs later	36.29	36.48	23.55	23.74
n/a	Healthy tomato root	40.00	40.00	40.00	40.00

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In this trial, it is not clear how uninoculated plots became infected, as substrate was largely enclosed in plastic, slabs were held separately above the ground, and plants were irrigated by dripper. A high level of inoculum was applied within the polytunnel, and it is possible some accidental spread occurred. One possibility is insects.

At the final assessment, where wrappers were removed from cubes and the cubes cut into pieces, a statistically significant difference was observed between treatments in root mat severity score in the cube (Figure 17). Additionally, significant differences were observed in the severity of symptoms present in the rockwool slab (Figures 18 & 19).

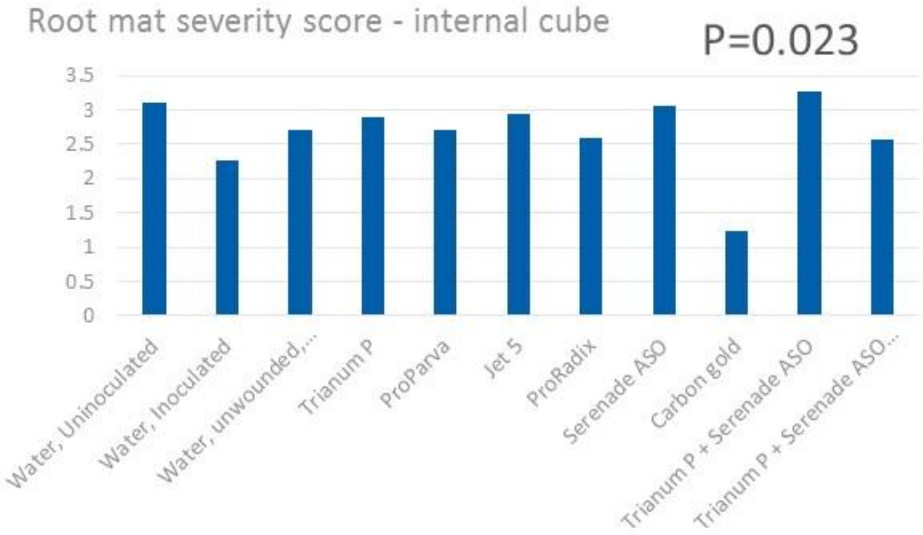

Figure 17. Root mat severity score (0-5 index) awarded to internal faces of propagation cubes at final assessment (LSD = 1.0164) - ADAS Boxworth, November 2016.

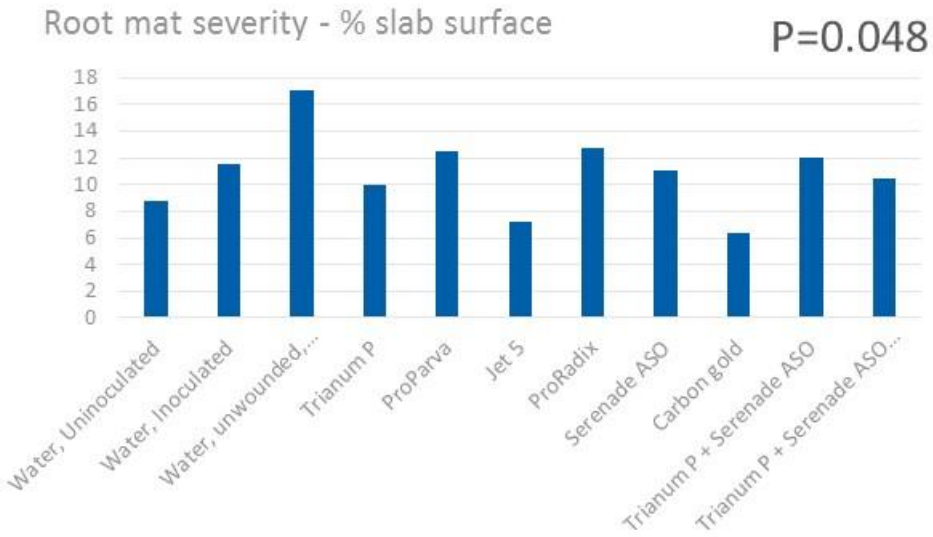

Figure 18. Root mat severity (% slab surface affected) awarded to rockwool slabs at final assessment (LSD = 5.718) - ADAS Boxworth, November 2016.

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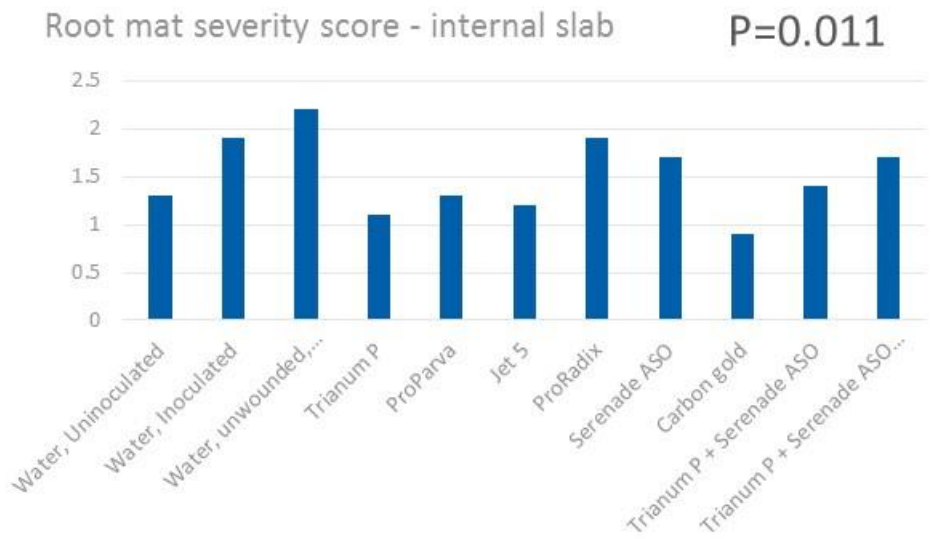

Figure 19. Root mat severity score (0-5 index) awarded to rockwool slabs at final assessment (LSD = 0.6884) - ADAS Boxworth, November 2016.

This revealed a more complex picture of root mat symptoms, as observable symptoms on the cube surface did not always tally with the symptoms observed within the cube and below into the slab. Interpretation was hindered as the untreated uninoculated plots were frequently assessed at similar levels of disease to treated plots. All data recorded at the final assessment is tabulated in the Appendix.

Though Carbon Gold did not appear effective in controlling root mat at earlier points in the trial, (resulting in one of the highest % cube surface affected), it was found to reduce symptoms within the cube and slab to a level significantly lower than in inoculated untreated plots. It was the only treatment found to significantly reduce levels within the cube (Figure 17). In terms of slab severity, Trianum P also significantly reduced levels, though it did not result in significant reductions in any of the other criteria assessed. When compared with the unwounded, inoculated treatment, the combined treatment of Trianum P and Serenade ASO, applied twice (Treatment 11), Jet 5 (Treatment 6) and Serenade ASO (Treatment 8) also significantly reduced % of the slab surface affected.

At the final assessment, roots sampled from each treatment tested positively for the presence of T-DNA. The fruit sampled from each treatment tested negatively for T-DNA. As such, it was thought unnecessary to also test seed.

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Additional experiments

Monitoring of root mat incidence and severity in difference coir substrate mixes

The incidence and severity of root mat on roots assessed in crops grown on the same commercial site in 2015 and 2016 are summarised below in Table 12. It is important to note that this was not a randomised trial, and the different types of coir slabs were located in different areas of the nursery, and also held different scion varieties. In 2016, two blocks with Brand 3 slabs were assessed separately, as incidence of root mat reportedly differed between two scion varieties. The variety exhibiting the most severe symptoms can be described as very vegetative, and it is reported by growers that more vegetative varieties commonly exhibit symptoms more severely.

Table 12. Summary of incidence and severity of root mat observed on a commercial site at the end of 2015 and 2016 seasons; note that all plants were grown on Optifort rootstock in both years.

Year **Coir type**	**Variety**	**Chip:pith ratio**	**Incidence (% cubes affected)**	**Severity (% cubes with severity score >3 on a 0-5 index)**
2015				
Brand 1	Amoroso	50:50	17.6	10.3
Brand 2	Amoroso	40:60	3.6	5.3
Brand 3	Piccolo	70:30	1.5	0.1
2016				
Brand 1	Piccolo	60:40	1.7	0.0
Brand 2	Bamano	70:30	1.0	0.0
Brand 3	Amoroso	70:30	23.3	10.6
Brand 3	Piccolo	70:30	7.5	1.5

These results point to both coir type and scion variety having a discernible effect on symptom expression. In 2015, Brand 3 slabs had markedly fewer notable symptoms, and cubes and slabs that could be described as being severely affected were largely absent. The worst symptoms were observed in Brand 1 slabs, which had a similar incidence of symptoms to slabs produced by Brand 2, but of notably greater severity. In 2015, it appeared that the greater chip:pith ratio of the Brand 3 bags influenced root mat symptom expression. It has been suggested that this is to do with the greater drainage and aeration capacity of slabs. A sample of the best (Brand 3) and worst (Brand 1) slabs were sent for substrate testing (Table 13). Additionally, symptoms observed on the site have always been worse in Amoroso, a very vegetative variety, than in Piccolo.

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Air filled porosity is the percentage of air spaces in a media after saturation with water and then being drained. Brand 1 slabs have slightly (approx. 5%) more air spaces than Brand 3. To put this in context, pure coir as a raw material can have an AFP of up to 20%, much lower than both these slabs (therefore these bags may have quite coarse coir, or have additional materials added). The higher the APF, the plants will have more aerated roots, but the media will drain more quickly and the plants will have to work harder to get water. The shrinkage value is about the same for both slab types, with slightly less shrinkage in the Brand 3, and about the same as has been recorded for raw material coir (approx. 20-25%). Below 1.5 kPa water is generally unavailable to plants, so the values for the pressure plate at 1kPa shows the percentage of water left available to the plant once 1kPa of pressure has been applied to the media. If this is too high the plant will become waterlogged. The Brand 3 has more water available to the plant under pressure than the Brand 1, though it is quite a small difference (approx. 2%), so whether it would translate into waterlogging is unclear.

These results do not explain the differences observed in root mat severity on the commercial tomato nursery, as in all aspects tested the bags did not differ by much. It is possible some other criteria not tested is contributing to the large differences observed in root mat severity.

Table 13. A summary of testing carried out on the best and worse coir bags in terms of root mat severity, sampled at the end of the season 2015 (three replicates of each bag)

Sample ID	AFP Score	Pressure plate			
		Shrinkage value	1kPa (-10 cm)	5kPa (-50 cm)	10kPa (-100cm)
			water volume	water volume	water volume
	(%)	%	%	%	%
Brand 3	40.54	21.54	43.10	34.38	34.03
	40.02	18.51	41.00	31.80	30.52
	43.77	19.80	43.50	34.99	33.92
Brand 1	46.99	21.20	40.69	32.08	30.52
	49.11	24.37	40.95	32.29	29.67
	48.59	20.41	40.33	32.08	31.34

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Commercial trial involvement, USA

The trial, over 16 commercial rows, was sown on 10th August 2016 and assessed on 12th September and 3rd October 2016. The results are summarised in Table 14.

Table 14. Incidence and mean severity of root mat on cucumbers cv. Verdon following implementation of potential control options - USA, 2016

	12th September		3rd October	
Treatment	**Incidence of root mat (no. cubes)**	**Mean severity of root mat (0-5 index)**	**Incidence of root mat (no. cubes)**	**Mean severity of root mat (0-5 index)**
1. Control	61.8	0.9	100	3.2
2. Prestop	62.0	0.9	99.3	3.0
3. Wrapper removal	62.5	1.0	100	3.4
4. Prestop + wrapper removal	69.5	1.1	99.3	3.3
p value	0.358	0.727	1.00	0.351

Symptoms can be seen to increase over time, but following analysis no significant differences were found in root mat incidence or severity between treatments. It appears in this case both wrapper removal at arrival on nursery and treatment with Prestop during propagation were ineffective in reducing root mat.

Discussion

Variability of isolates & implications of a new diagnostic tool

Combined findings from genome sequencing of pathogen strains and pathogenicity testing in the greenhouse have confirmed that, although genetically variable, all rhizogenic isolates from UK cucumber and tomato root mat are *Rhizobium rhizogenes* carrying cucumopine Ri plasmids. Other *Rhizobium* species carrying different Ri plasmids have never been isolated from UK tomato or cucumber crops. Furthermore, available diagnostic methods based on conventional PCR (Haas *et al*., 1995) and TaqMan qPCR (Weller and Stead, 2002) were confirmed to detect common T-DNA targets from the Ri plasmids of all UK root mat isolates. In addition, detection of T-DNA in tomato root samples was demonstrated even before root mat symptom development was apparent. With the introduction of a DNA extraction and purification method that allowed direct testing of plant roots without the need for prior bacterial enrichment, it is now hoped that a test can be fully validated for screening of propagation material prior to transplanting. This would permit detection of infected roots, even in the absence of the bacterium that initially infected the plant.

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Effect of inoculum concentration - is there a 'threshold' for infection?

Pathogenicity testing at Fera confirmed that rate of symptom development was related to the initial inoculum concentration under constant environmental conditions. Even under conditions conducive to disease development (constant 25°C and 75% RH), a high inoculum threshold (equal to or greater than 10^6 cfu per ml or 1.5 x 10^7 cfu per plant), applied to wounded roots, was required to induce root mat symptoms within a 5 week period. These findings agree with those observed for crown gall, where a high threshold population of *R. radiobacter* is needed to induce quorum sensing controlled pathogenicity. Such results imply that any treatment with potential to suppress or prevent multiplication of natural primary inoculum sources should have a high probability to slow down or even prevent root mat development. However, the findings that the bacteria appeared to spread readily from infected to healthy plants during various experiments suggest that once initial infection is allowed to occur it may be difficult to control further disease development.

Efficacy of products

In the product screening trial at ADAS Boxworth, it is possible that as tumour inducing *R. radiobacter* inoculum used was of a high concentration, more subtle differences in disease incidence due to treatments were masked. Incidence of root mat symptoms was largely unaffected by the treatments applied, but the product Proradix was observed to reduce the incidence of symptoms on the cube surface. This may be because the Pseudomonas species contained in this product has been shown to effectively colonise roots in competition with other soil bacteria (in barley; Buddrus-Schieman *et al.,* 2010) and to aid mycorrhisation in tomato (Yusran *et al.,* 2007), which may allow some protection from initial infection. Additionally, the severity of symptoms observed on the surface of cubes and the severity of symptoms within the cubes and on the slabs were not as related as one might expect. This may be because cubes were inoculated by drench, and symptoms were able to develop easily around the point of inoculation. However, some treatments may have been able to successfully delay disease development/spread into the cube and slab more effectively than others. The treatments which appeared most adept at this suppression were treatment with Trianum P and Serenade ASO (therefore inoculating two beneficial organisms at a time) and Carbon Gold Biology Blend (also containing multiple microbial ingredients). A mixed microbial inoculation may be more likely to effectively suppress the action of *R.* radiobacter as a greater microbial biodiversity can make a population more resistant to extreme shifts in its make-up, essentially preventing *R. radiobacter* from becoming dominant in the system.

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Effect of wounding

It is known that root infection by *Rhizobium radiobacter* carrying tumour-inducing (Ti) plasmids is encouraged by wounding, which releases phenolic compounds that are attractive to the bacteria. It therefore seems likely that the same process will encourage initial infection by the same species carrying Ri plasmids. In the product screening trial at ADAS Boxworth, tomato plants grown without any imposed wounding (seeds sown onto cubes) developed root mat to the same extent as plants where roots were cut with a scalpel before inoculation. This indicates that natural wounds, such as those that occur where lateral roots emerge, are sufficient to allow root mat infection in tomato. In work on crown gall disease, it was shown that infection of direct-sown walnut seedlings occurred around lateral root emergence sites (Yakabe *et al.,* 2012).

Effect of substrate

Presently, there is discussion on the effects of substrates on root mat disorder, especially regarding the differential qualities of substrates to hold water and oxygen. Though root mat symptoms were observed to differ between slabs with different chip:pith ratios on a commercial site, when slabs were tested they were not found to differ significantly in key substrate properties. It is therefore likely that some other aspects on site are affecting root mat incidence (e.g. scion variety, inoculum load of surroundings) or that the substrates do differ in some way not examined. It has been noted that root mat symptom expression is linked to moisture in the substrate, with some growers preferring to remove plastic wrappers from cubes or slabs to facilitate quicker drying of the substrate. It is certainly true that in the inoculation trial at ADAS Boxworth, where plants were grown in open trays that continued to hold any run-off, symptoms expressed were very noticeably worse than in the product screening trial where excess irrigation solution was allowed to run-off from slabs. However, in a replicated trial on a commercial site (in the USA) removing the plastic wrapper from propagation cubes was observed to have no effect on root mat incidence and severity. The percent water content in propagation cubes or slabs was measured throughout both the trials (using equipment courtesy of Grodan), however as this was not manipulated as part of a treatment, effects are difficult to define. In the inoculation trial, the water content was largely dictated by the two different root systems present (grafted to a rootstock or on the scion varieties roots), but symptoms were first noticed after a large increase in water content, and again expressed symptoms climbed rapidly following another large increase. Similarly, in the product screening trial, an increase in symptom expression corresponded with a general increase in water content in the slab over the course of the trial. Graphs of percent water content can be seen in the Appendix.

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These investigations were additional to the original objectives proposed, as a response to discussion with the industry, and it is likely that for more definitive data on these aspects of root mat control, specifically designed trials would have to be carried out.

Conclusions

- There are a number of knowledge gaps regarding *R. radiobacter*, the Ri plasmid and the transfer of T-DNA.
- Fera are in possession of a molecular assay capable of confirming the presence of T-DNA in plant roots.
- This test is not dependent on presence of *R. radiobacter* and can therefore bypass the enrichment step used in the conventional diagnostic, potentially making the test faster and less expensive.
- Whole genome comparisons showed that UK isolates of *R. radiobacter* biovar 1 are highly heterogenous.
- A high population density of rhizogenic *R. radiobacter* is required to initiate root mat consistently.
- Tomato plants at 19 days old and 33 days old were successfully inoculated, and both grafted and ungrafted plants appeared equally susceptible.
- First symptoms were noted approx. 3 weeks after inoculation, but it is unlikely these would be apparent in a commercial setting; more notable symptoms appeared in the inoculation trial 6-7 weeks after inoculation.
- A number of non-conventional products applied to the rootzone were observed to reduce the expression of root mat symptoms.
- The most effective treatments tested were Carbon Gold (biology blend) and a mixed treatment of Trianum P and Serenade ASO, when applied both before and after inoculation.
- A product containing a *Pseudomonas* sp. known to colonise tomato roots also appeared to reduce incidence of root mat disease.
- The effect of imposed wounding on infection remains unclear, and disease severity did not appear to differ between wounded and unwounded plants; this suggests that natural wounds, such as at points of lateral root emergence, are sufficient for infection.

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Knowledge and Technology Transfer

Presentation to The Tomato Study Group, Fenstanton, Cambs, 18 October 2016 (S Mayne).

Article in AHDB Grower magazine, in preparation.

Glossary

Biovar – the name applied to a population distinguished on the basis of biochemical or physiological properties

Opines – low molecular weight novel metabolites synthesised in plant tissues following incorporation of plasmid DNA into the plant genome; over 30 different opines have been described. They are amino acid derivatives used almost exclusively by bacteria as a source of carbon and nitrogen.

Plasmid – a genetic structure in a cell that can replicate independently of the chromosomes, typically a small circular DNA strand

Quorum sensing – a signalling system between bacteria

Rhizogenic – root inducing

T-DNA – transfer DNA; the section of a plasmid transferred into a plant cell and incorporated in the plant genome

Tumorigenic – tumour inducing

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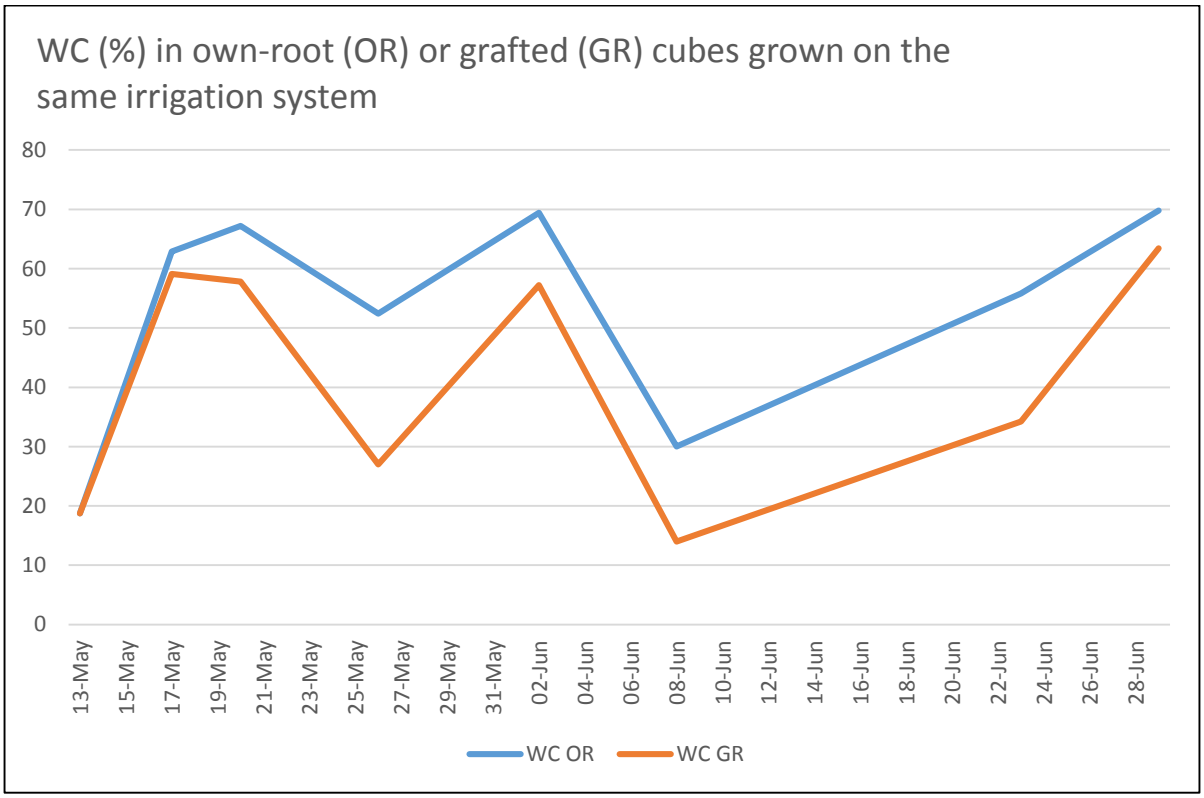
Appendices

Additional information relating to Boxworth inoculation trial - Spring 2016

Water content of substrate



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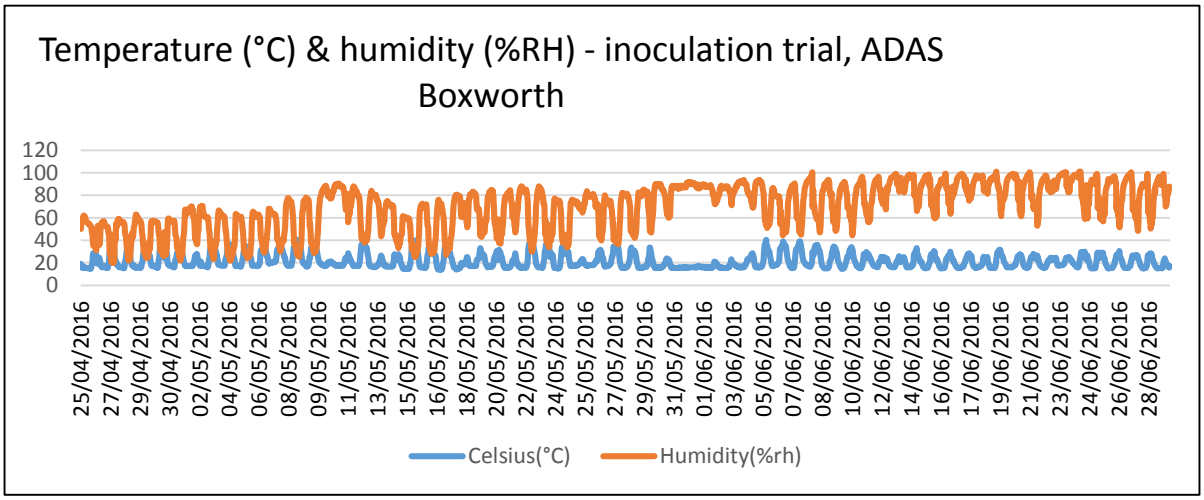
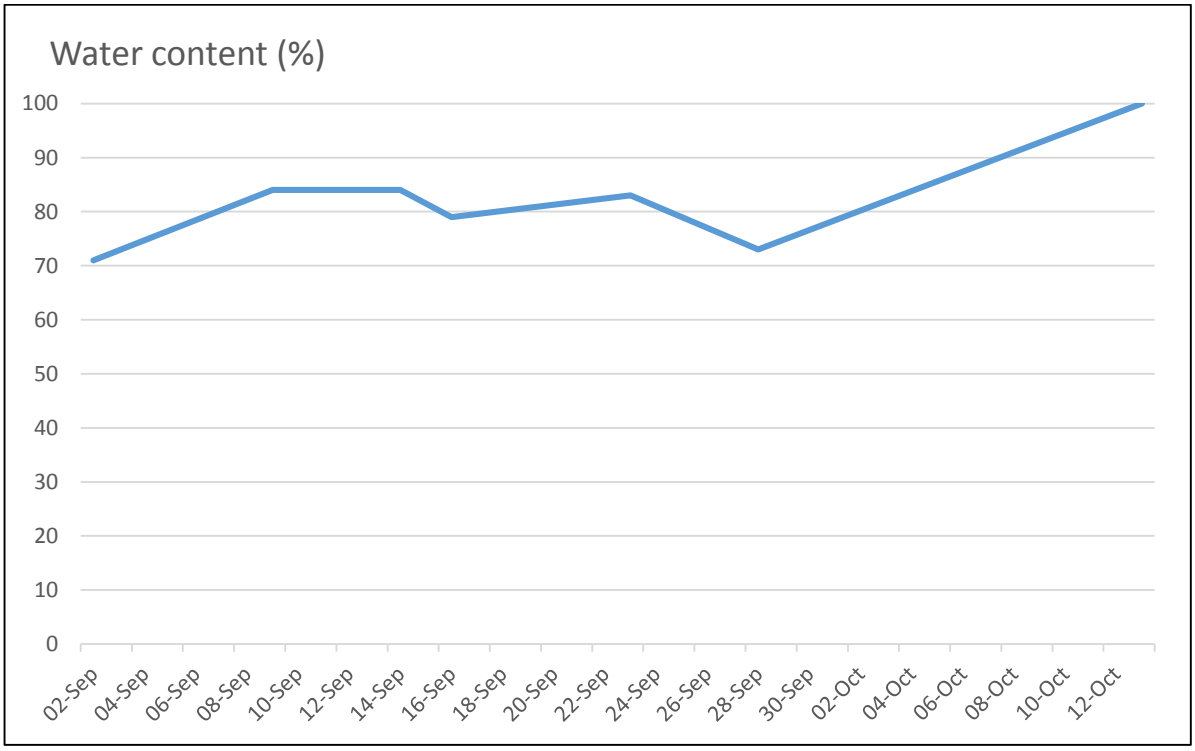
Environmental data



Additional information relating to Boxworth product screening trial - Summer 2016

Water content of substrate



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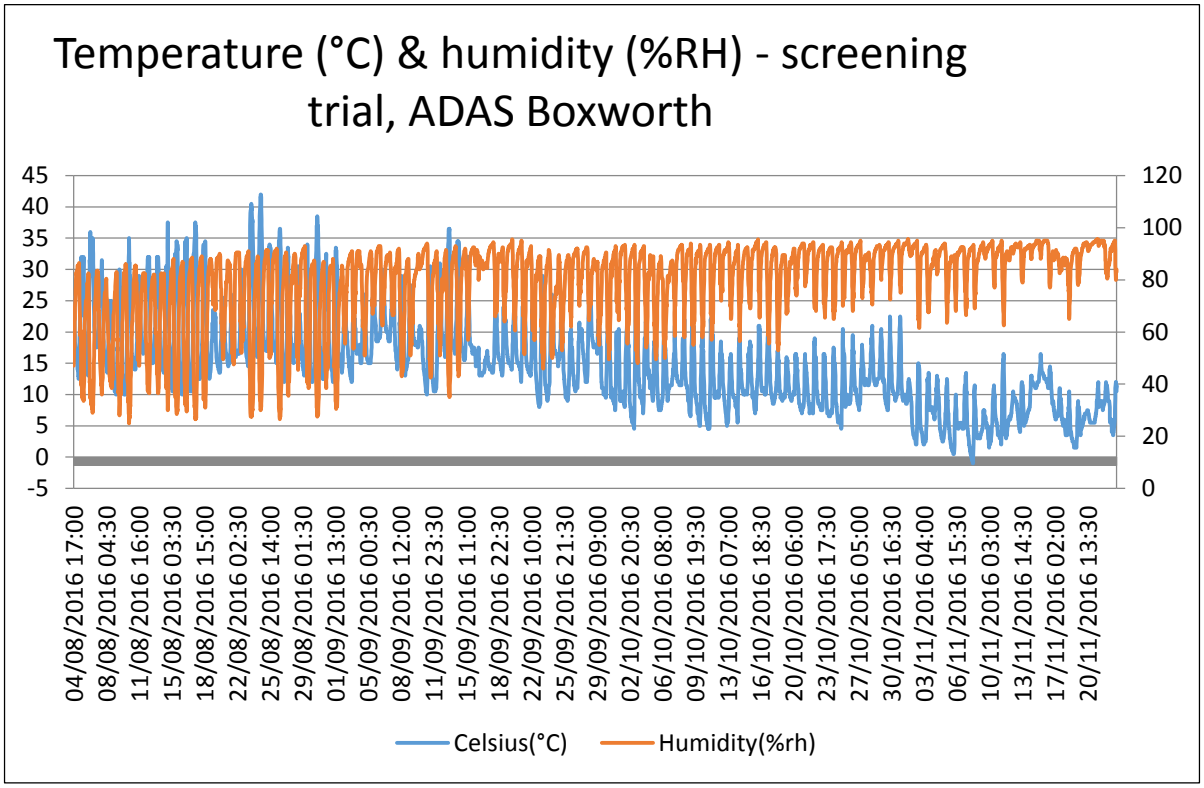
Environmental data



Final assessment data

Trt		Vigour	Cube incidence	% cube surface	Internal cube severity score	% slab surface	Internal slab severity score
1	Water, Uninoculated	4.6	96.67	9.47	3.1	8.8	1.3
2	Water, Inoculated	4.6	100	7.67	2.267	11.5	1.9
3	Water, unwounded, inoculated	4.9	100	10.93	2.7	17	2.2
4	Trianum P	4.967	100	12.87	2.9	10	1.1
5	ProParva	5	100	12.4	2.7	12.5	1.3
6	Jet 5	4.933	96	10.13	2.933	7.2	1.2
7	ProRadix	4.733	90	8.63	2.6	12.7	1.9
8	Serenade ASO	4.967	100	10.47	3.067	11.1	1.7
9	Carbon Gold	4.867	96.67	11.07	1.233	6.4	0.9
10	Trianum P + Serenade ASO	4.967	96.67	8.2	3.267	12	1.4

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11	Trianum P + Serenade ASO and again 24 hours later	4.633	92.67	5.67	2.567	10.5	1.7
p value		0.303	0.288	0.112	0.023	0.048	0.011
LSD		0.4123	8.608	4.652	1.0164	5.718	0.6884

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