



# Grower Summary

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## **PE 029**

Protected tomato: Evaluation of biological treatments, biocides and an improved diagnostic for control of root mat disease

Annual 2016

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**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

**Report:** Annual report, December 2016

**Previous report:** None

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**Location of project:** ADAS Boxworth & commercial sites

**Industry Representative:** Dr Phil Morley, TGA & APS group  
Phil Pearson, APS group

**Date project commenced:** 1 January 2016

**Expected completion date:** 30 December 2018

## **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 Triatum 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-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.

**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) 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 conjunction

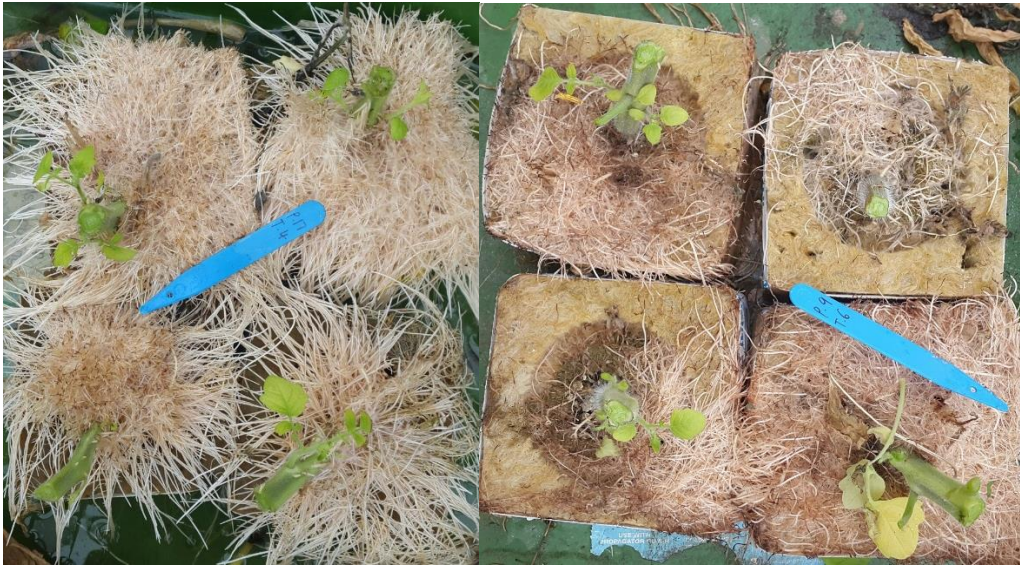
with pathogenicity testing on tomato seedlings in the greenhouse, confirmed all those able to cause root mat (rhizogenic) on tomato or cucumber as *Rhizobium radiobacter* carrying a particular root inducing (Ri) plasmid, known as a cucumopine Ri plasmid. Not all isolates from tomato or cucumber with 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 (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.



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

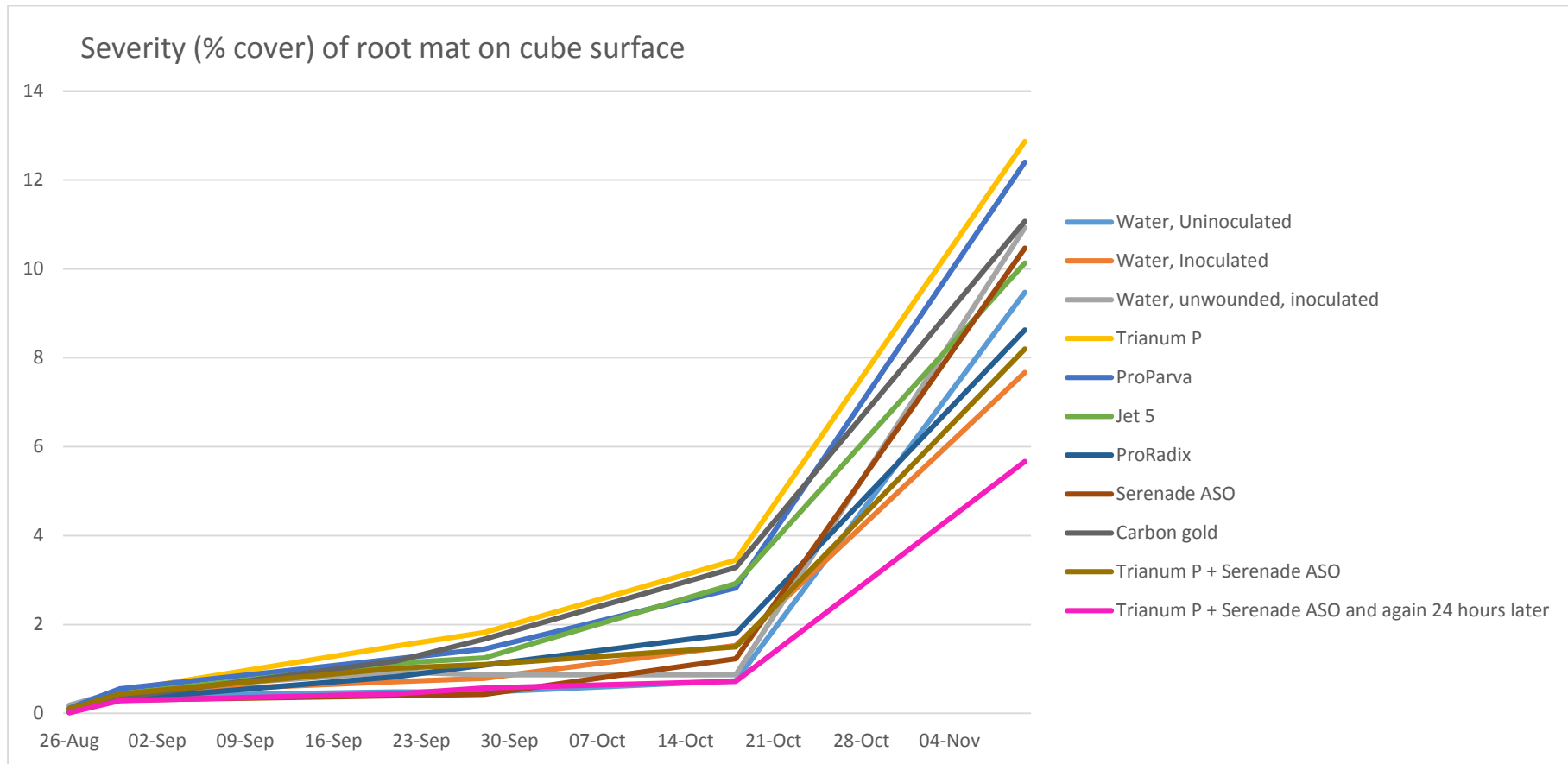
Following this trial, ungrafted Elegance was selected for use in a larger trial to test a variety of non-conventional products for their ability to control root mat on tomato. This trial was set up using commercial rockwool slabs, with plants grown on for 14 weeks after inoculation. Each plot contained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

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

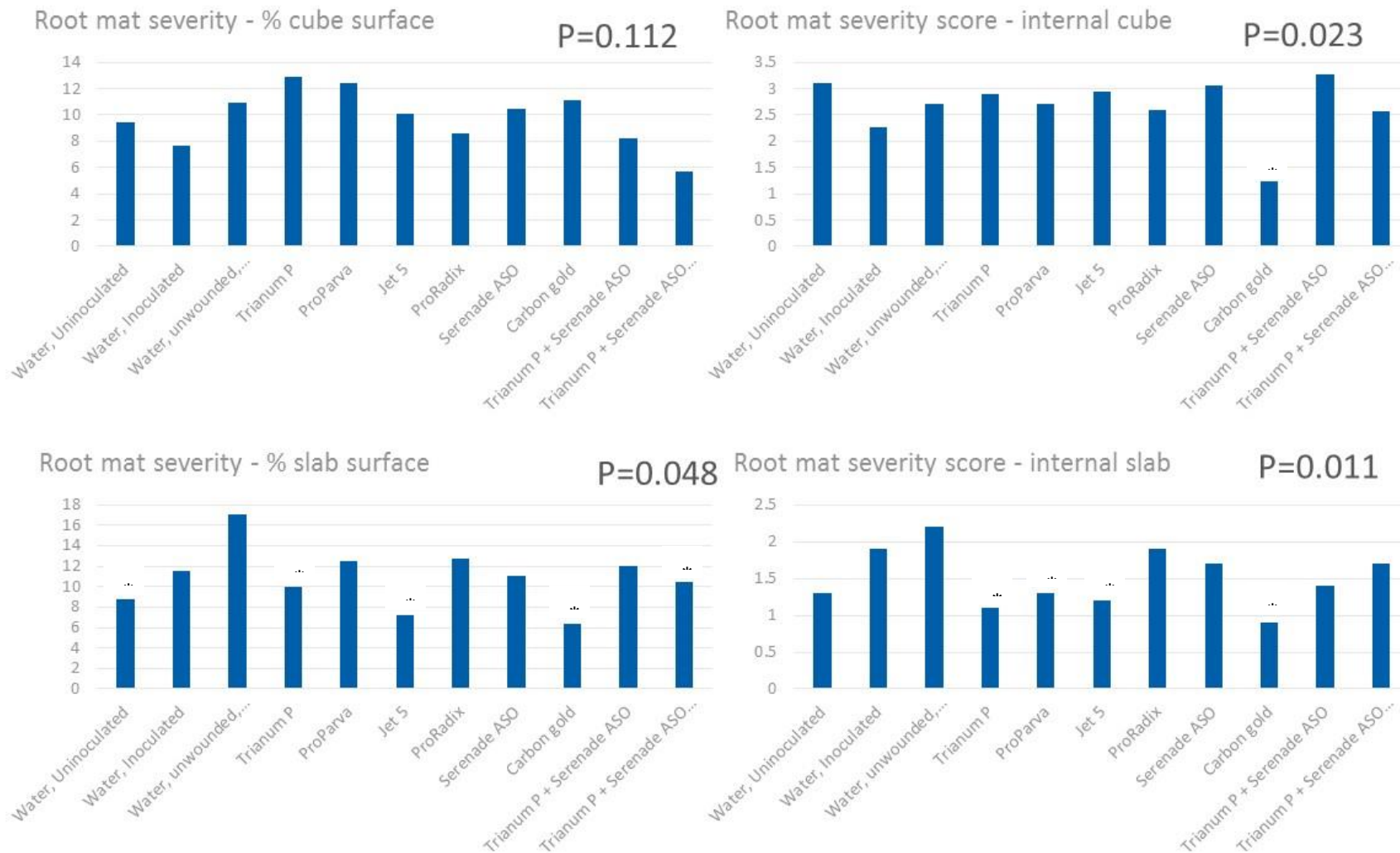
Treatment	Product	a.i.
1	Untreated uninoculated	water
2	Untreated inoculated	water
3	Unwounded inoculated	water
4	Triatum P	<i>Trichoderma harzianum</i> T-22
5	ProParva	Plant auxins
6	Jet 5	Hydrogen peroxide
7	Proradix	<i>Pseudomonas</i> sp. DSMZ 13134
8	Serenade ASO	<i>Bacillus subtilis</i> QST 713
9	Carbon Gold Biology Blend	Enriched biochar, microbes, wormcasts, seaweed etc.
10	Triatum 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).



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



**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).

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.

#### **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.