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:	Dave Kaye, ADAS
Report:	Final report, December 2018
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Location of project:	ADAS Boxworth, three commercial sites and Fera Science Ltd.
Industry Representative:	Dr Phil Morley British Tomato Growers' Association (Technical Officer) APS Group (Director of Agronomy)
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The results and conclusions in this report are based on an investigation conducted over a three-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.

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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CONTENTS

GROWER SUMMARY

Headlines

Diagnostics:

- A molecular diagnostic test validated during 2016 was used in 2017-18 trial work to identify tomato roots genetically modified (transformed) by the incorporation of the *Rhizobium* Ri plasmid (pRi) DNA into the plant cell's genome; this confirmed infection by the plasmid responsible for causing root mat symptoms
- Symptomless plants infected with pRi were detected at plant arrival on grower holdings, suggesting infection occurred during propagation or transport
- Symptomless plants infected with pRi were also detected within crops. The first visually symptomatic plants were observed around 8-16 weeks post-planting
- Infection was not detected in seeds tested from the propagator

Treatments:

- A biological approach reduced root mat disease in glasshouse trials in 2016 and 2017
- Carbon Gold—an enriched soil and substrate additive—applied at propagation alone, and at both propagation and planting, reduced the incidence and severity of root mat disease in two of the three trials in 2017
- Carbon Gold treated at propagation alone reduced the incidence of root mat disease by 50% in one trial in 2017
- A greater incidence of root mat disease was recorded in plants grown on once-used coir slabs with a history of root mat disease than on new, unused coir slabs
- Chlorine-based biocides were significantly more effective than hydrogen peroxide-based biocides at killing some strains of *Rhizobium radiobacter* at the concentrations and exposure times tested
- A newly formulated sodium hypochlorite product (Domestos Extended Power) was the only product tested that eliminated viable *R. radiobacter* in biofilms on irrigation tubing within the experimental conditions used
- A chlorine dioxide-based biocide (Clorious2) effectively killed *R. radiobacter*, as cells suspended in water, at concentrations that are known not to be phytotoxic or corrosive

- Removal of the plastic surrounding the propagation cubes and / or removal of the plastic from the surface of the slabs did not reduce root mat disease incidence or severity in a trial conducted in 2018
- Biofilms can form within the irrigation system and may allow for early infection of clean, young susceptible plants following plant arrival

Background

Root mat disease in tomato is an increasing problem both in the UK and around the world. The disease is caused by strains of *Rhizobium radiobacter* which carry a root-inducing (Ri) plasmid. These bacteria genetically modify (transform) tomato root cells through the incorporation of this plasmid DNA into the host plant cell's genome. Crop observations over two years of commercial trials as part of PE 029, combined with current knowledge of root mat disease biology, suggest that infection occurs in young plants at grower sites, or at propagation. It can then take several weeks to months before visible symptoms manifest. Root samples collected immediately at plant arrival on one site in 2018 tested positive for infection, confirming infection had occurred by this time.

Root mat infection leads to excessive vegetative growth and reduced fruit size and quality, whilst increasing the risk of secondary root rot development. Infections are generally sporadic in nature; often one cube will exhibit severe infection, whilst the neighbouring cube is completely free of visible symptoms. However, it may be infected and asymptomatic of the disease. This has an impact on crop uniformity and negatively affects the ability of growers to effectively steer infected crops. Combined, these effects can result in significant crop losses estimated at 15% (including added management costs).

There are no proven treatments for complete disease control, however the use of biological treatments has shown some promising results. Current control measures focus on biological treatments, crop management and hygiene; there are no approved bactericides. An increasing number of biological products reported to assist optimisation of plant health and / or resistance to disease are now available. For example, the NatuGro programme is used widely, though there is no evidence for its effectiveness against root mat.

Summary

This project concentrates on management of root mat disease by both prevention of infection and reduction of subsequent symptoms. In the first year of the project, current knowledge was reviewed (Objective 1), an improved diagnostic test was developed (Objective 2), and the efficacy of a number of biocontrol products were examined in trials at ADAS Boxworth

(contributing to Objectives 3 & 4). The efficacy of several commonly used biocides at crop turnaround, in reducing populations of *R. Radiobacter* was also established (Objective 5).

The project's specific aims and objectives 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 plant roots by bacteria carrying the root initiation plasmid (pRi); to develop a rapid molecular test for early detection of infected plants.

(ii) 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 AHDB 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;
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.

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 (Years 1 and 3).

The review was completed in 2016 and can be accessed in its entirety here and via the AHDB Horticulture website. A factsheet on tomato root mat is in preparation.

An additional review update has also been produced as part of the final report from AHDB project CP 174, on *Rhizobium* spp. causing hairy root and crown gall disease; this is also available on the AHDB website here.

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 (Year 1).

A rapid molecular test was validated in 2016 against several isolates of *Rhizobium* from UK tomato and cucumber crops by Fera Science Ltd. This included additional reference strains known to cause similar root proliferation in different crops. This new DNA extraction method

allows for the direct detection of the T-DNA sequences in plant roots. This was compared with an existing test involving 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. Full details of this work can be found in the PE 029 2016 annual report.

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 - 2017 & 2018.

In 2016, a preliminary trial was established to determine an effective inoculation method using inoculum generated by Fera Science Ltd. Symptoms were produced in grafted and ungrafted plants. Infection occurred in plants inoculated at the plug stage following wounding by rough handling at transplant. Symptoms also developed in seedlings which were inoculated two weeks later, following wounding. Root mat infection was confirmed via qPCR (Objective 2).

A follow up trial tested eight non-conventional products for their ability to control root mat in tomato. Of the eight treatments tested, Carbon Gold, Trianum P and Serenade ASO reduced root mat disease. Full results can be found in the PE 029 2016 annual report. Further work on this objective carried out in 2017 and 2018 is reported under Objective 4.

Objective 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 – 2017 & 2018.

2017 Trials

Three large commercial trials—Trials A, B and C—were undertaken in 2017 to further examine post planting treatments (Objective 4) and product efficacy (Objective 3). Carbon Gold was chosen based on its performance during 2016, and Vitix was chosen as an alternative product to Serenade ASO, which is not approved as a drench treatment for use on protected tomato.

Plants were treated at propagation, at both propagation and in crop, and in crop alone. Carbon Gold, an insoluble biochar substrate additive containing beneficial microorganisms, was applied once at propagation and once at planting. Vitix, a mixture containing plant growth promoting micro-organisms (*Trichoderma* spp. and *Bacillus* spp.), is water soluble and was applied by hand every eight weeks.

Root samples from the three propagation treatments were tested at plant arrival at Sites 1 and 2. This revealed that treated plant material was infected with root mat disease at this time. Seed was also tested from the propagator and returned negative for infection.

Plants for Trials A (cv. Piccolo) and C (cv. Funtelle) were grown on new coir slabs, whilst those for Trail B (cv. Piccolo) were grown on once-used coir slabs with a history of root mat disease.

Crops were assessed, at first symptom detection, mid-cropping (July–September) and at end-of-cropping (November) for incidence and severity of root mat disease. Additionally, root samples were taken at the first and final assessments from cubes asymptomatic and symptomatic of root mat disease to confirm symptoms and to examine disease spread. Despite a low disease incidence, a statistically significant increase in root mat incidence was seen in plants treated with Vitix at propagation alone in Trial A, at the first-symptom assessment. This was accompanied by an increase in severity scores of cubes exhibiting greater than 10% of surface root mat coverage. This pattern of increased incidence and severity continued to the final assessment.

At the first symptom assessment of Trials B and C, no differences in disease incidence or severity were found between any of the treatments and the untreated control. At the mid-crop assessment in Trial B, a treatment of Carbon Gold at both propagation and planting reduced the incidence of root mat disease to 33% (compared to 43% in the untreated)—a commercially useful reduction. An additional positive treatment effect was a reduction in the proportion of cubes suffering a severity score of greater than 10% of cube surface coverage in Trial B. All treatments except Vitix applied in propagation alone gave reductions, with Carbon Gold applied at both propagation and planting and Vitix applied at both propagation and in crop giving the greatest reductions in disease severity.

Carbon Gold applied at both propagation and planting in Trial B reduced the incidence of root mat disease to 44%, compared to 54% in the untreated, by the final assessment. At this assessment in Trial C, Carbon Gold applied at propagation alone reduced the incidence of root mat by half, to 16.8% compared to 34.8% in the untreated plots. Carbon Gold applied at propagation alone and the same product applied at both propagation and planting also reduced the severity of the disease by significant amounts—but to a lesser extent.

Carbon Gold treatments at propagation alone, and at both propagation and planting, were shown to have an effect. This suggests that application at propagation is an important treatment window, but it may be necessary to repeat the treatment at planting to see an effect. A full breakdown of the results from Objectives 3 and 4 for 2017 is included in the PE 029 2017 annual report.

2018 Trials

Three commercial trials were carried out in 2018—Trials D, E and F. Trials D and E furthered work carried out in 2017, aiming to identify the optimal time for Carbon Gold application. A third trial—Trial F—examined the effect on root mat disease of removing the plastic sleeve surrounding the propagation cube and / or the plastic on the surface of the slab.

Despite the large size of the two trial areas, very little visible root mat developed over the 2018 season, with just one case of the disease occurring in Trial D and five cases in Trial E. This low incidence of root mat disease was unexpected, especially considering the severe infections which were present on Site 2 during 2017.

The plants used in Trial D were sourced from a UK based propagator, whilst those for Trial E were sourced from a Dutch propagator. Root samples collected before planting were sent for qPCR analysis. The results showed low levels of infection were present in the plants treated with Carbon Gold at propagation in Trial D. All samples tested in Trial E returned positive for root mat infection with moderate to high levels of infection detected. Unfortunately, with such low levels of infection present, it was not possible to perform any meaningful statistical analysis on the data gathered in Trials D and E at any date. It is unclear why levels of root mat disease did not increase, especially when the plants used in Trial E were infected at arrival.

Trial D was continually dosed with low levels of Clorious2 through the irrigation system; this may have restricted *R. radiobacter* population growth, preventing infection from reaching a level where visible symptoms developed. However, continual dosing of Clorious2 was unable to completely prevent infection from occurring in the trial area, or within non-trial (untreated) rows.

Trial E used the NatuGro programme across the site, with dosing of biologicals throughout the year. There is no direct evidence that the NatuGro system has a positive effect in reducing root mat disease incidence. It is possible that the specific strain of *R. radiobacter* present was not as aggressive, or the environmental conditions experienced during the prolonged sunny weather of 2018 impacted the activity of the pathogen. Further work would be needed to examine this.

Significant work has been performed on different *R. radiobacter* strains, and variation between individual strains has been shown to exist; it is possible that these strains may react differently to different control methods. Strains have been demonstrated to grow at a wide variety of temperatures and pH values, and some can form biofilms (Bosmans L, 2015). Some strains are catalase positive, enabling them to convert hydrogen peroxide at concentrations

above the shock treatment recommended by manufacturers in some cases (Bosmans L, 2017).

The positive beneficial results using Carbon Gold in 2017 occurred in Trials B and C, based at Site 2. It is plausible that the same *R. radiobacter* strain was present in both trials and was successfully controlled by one, or several, of the components within Carbon Gold. The plants at Site 1 in 2017 were sourced from a different propagator and were likely infected with another strain which may have responded differently. This may explain the negative reaction to Vitix and the lack of positive disease control in cubes treated with Carbon Gold in Trial A.

Plastic removal trial

Many growers choose to remove the plastic surface from the top of their slabs to assist the management of root mat disease; there is a belief that this may suppress disease symptoms. In Trial F, two treatments were compared to 'untreated' slabs – the removal of a cube's plastic sleeve, and the removal of a cube's plastic sleeve and the plastic covering the slab's surface. The ability of these treatments to manage root mat disease on tomato (cv. Sunstream) was assessed; Trials D and E were assessed concurrently.

Overall disease levels were low, and at each of the three assessments, one plot contained the majority of the disease occurrences – this data was excluded from the analysis. Removal of the plastic wrapper and / or removal of the plastic sleeve was shown to have no positive effect in reducing the incidence or severity of root mat disease. There is potential that an effect could have been seen if greater levels of root mat disease was present. To test this, further work repeating the trial under greater disease pressure would be required.

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

The efficacy of four biocides—Clorious2, Geosil, EndoSan3 and a newly formulated Domestos product (Domestos Extended Power)—were compared in laboratory conditions. These biocides were tested for their ability to kill pure cultures of *R. radiobacter* at different doses and exposure times (Experiment 1), and their ability to remove biofilms of *R. radiobacter* from PVC irrigation piping (Experiment 2).

Experiment 1 – Biocide efficacy testing on pure R. radiobacter cultures

Each biocide was added to suspensions of *R. radiobacter* at a known concentration (10^7 CFU / ml), at five concentrations of the products' recommended doses. Once mixed, a subsample was removed after four different exposure times (0, 2, 5 and 10 minutes) and grown onto nutrient dextrose agar at 28°C for 48 hours. Growth of any surviving bacteria was recorded.

The most effective biocide tested was Domestos Extended Power, achieving 100% kill of the bacterium at all concentrations tested. Clorious2 was 100% effective at half and full

recommended doses, but only if the exposure time was 2 minutes or greater. No effect of Clorious2 was observed at 0.1x the recommended dose. The recommended dose of Clorious2 (0.75 ppm chlorine dioxide, ClO_2) has been found to be non-phytotoxic to tomato plants, whereas the recommended dose of Domestos has a significantly higher active chlorine concentration and is only recommended for disinfection of surfaces. Suggested practical application of Domestos at this rate would therefore be limited only to treatment of surfaces in the absence of growing plants, followed by thorough flushing out of the product prior to any subsequent contact with plants (such as at site clean-up). Domestos is not currently licensed as a biocide for this purpose however.

The hydrogen peroxide (H_2O_2) / stabilised silver biocides Geosil and EndoSan3 had no effect on *R. radiobacter* at their recommended rates, or at 2x these rates. Some effect was observed, which increased with exposure times above 5 min at 5x the recommended rates. This lack of efficacy was later attributed to catalase enzyme activity by the strain of *R. radiobacter* used, enabling the conversion of the active hydrogen peroxide to water and oxygen, effectively nullifying the effects of these products at the concentrations used.

Experiment 2 – Efficacy testing of the ability of different biocides in treating biofilms of R. radiobacter.

Segments of PVC dripper tubing (1cm long) were coated in bacterial biofilm containing cultures of *R. radiobacter*. Each biocide was prepared at the shock treatment concentrations of ClO_2 and H_2O_2 recommended by the manufacturers for surface disinfection. Contaminated tube segments were submerged in each biocide and exposed for 2, 5 or 10 minutes. Enrichment of any remaining viable bacteria was performed by assessing any bacterial growth before and after enrichment in selective broth media as indicated by quantitative TaqMan (qPCR) analysis.

Domestos Extended Power alone was effective in preventing further multiplication of *R. radiobacter* at the concentrations and exposure times tested. Testing of the concentrations of ClO_2 and H_2O_2 active ingredients indicated that there were detectable residual levels at the end of each exposure time, suggesting that some of the bacteria in the biofilms remained protected from biocide activity, rather than there being insufficient active ingredient present. The recently reformulated Domestos product appeared to effectively penetrate the whole biofilm, eliminating all viable bacteria, even within the first 2 minutes exposure (as indicated by no significance between pre- and post-enrichment Ct values). This Domestos product may therefore be a good candidate for end of season flushing of the irrigation line. However, adequate flushing with clean water must be carried out to remove any phytotoxic hypochlorite residue. Further investigation will be needed to determine whether the other biocides can

successfully remove viable biofilm by increasing doses and/or exposure times. A full description of the work carried out in Objective 5 is included in the PE 029 2017 annual report.

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

During 2018, ongoing project work was presented at two Tomato Working Party meetings (January and November 2018) and one Tomato Study Group Meeting (September 2018) to members representing a large proportion of UK tomato production. The results were also shared at a Protected Edibles Vine Crop Research Day in February 2019.

Additional observations

During 2017, an additional area of a Vitix treated crop was assessed at Site 2. Differences were seen in the distribution of disease incidence which corresponded to the irrigation system design. These observations were anecdotal in nature but provide evidence that the irrigation system may play a role in the location of root mat disease occurrence.

During 2018, at Site 3 the irrigation system feeding Trial F was scrutinised following the unexpectedly high amount of root mat disease found in plot 21. In the glasshouse containing the trial, the irrigation supply system splits following a solenoid valve, with one pipe feeding plots 1-20 and another pipe feeding plots 21 and above. At the first symptom assessment, twelve cases of root mat disease were found, ten in plot 21 and two in plot 23 (Figure 1.).

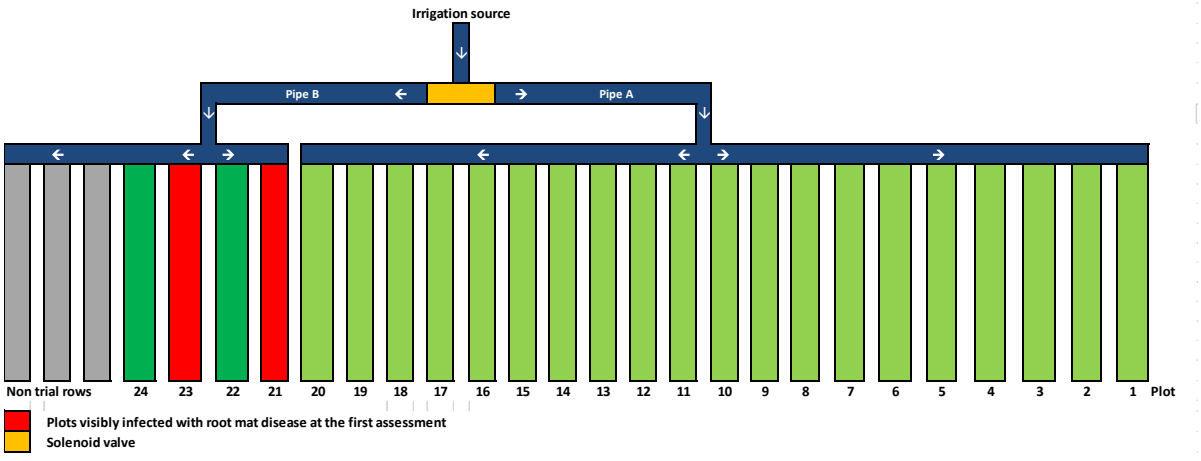

Figure 1. Distribution of root mat disease at the first symptom assessment, Trial F, Site 3 – April 2018

Roots in Trial F tested negative for root mat infection at planting. This result and the distribution of infection at the first assessment suggests that infection occurred from a pre-existing source, potentially the irrigation system. In 2017, Objective 5 demonstrated that none of the trialled biocides—apart from new formulation Domestos—were able to completely

eradicate pure biofilms of *R. radiobacter* from the PVC pipe at the concentrations and times tested. Clean-up at the end of the season may have been sufficient to eliminate or suppress levels of *R. radiobacter* to negligible levels in plots 1-20, but not plots 21 and above. Persistence of biofilms beyond the solenoid valve, or within the pipes feeding individual plots, including plot 21, could explain this disease pattern.

Drip pegs were sampled at each of the three sites from cubes demonstrating severe root mat infection, to determine if *R. radiobacter* was present. Viable *R. radiobacter* + pRi was isolated from all. It is possible that *R. radiobacter* can survive between crops, especially where turn-around times are short. Reuse of insufficiently disinfected pegs could allow for infection of uninfected plants following arrival, at a time where plants are highly susceptible to infection.

In cases such as Trial F, where plants are delivered free of root mat infection, it should be possible to eradicate tomato root mat disease from a site through complete and thorough clean-up practices, using appropriate biocides and disinfectants. However, a comprehensive clean-up will be immediately undone where infected material is delivered to sites.

Growers could request that propagators sample roots prior to the dispatch of tomato plants to their sites, or sample immediately at arrival to determine if infection has been introduced. This information could be used to develop root mat management strategies based on knowledge of which batches are infected and an estimate of the levels of infection. Isolates of *R. radiobacter* could also be tested to determine if they are catalase positive and able to tolerate hydrogen peroxide biocides—this may inform growers on the most appropriate biocides to use to manage tomato root mat disease.

Financial Benefits

The financial benefits from even partial control of root mat can be significant because losses can be very large:

- Consequential losses and additional costs due to the presence of root mat disease on one UK nursery were 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.
- Often, root mat does not affect all plants in a crop evenly, hence crop steering becomes increasingly difficult as symptoms appear and the previously homogenous crop profile becomes 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.
- As *R. radiobacter* is ubiquitous in the environment, good hygiene and sanitation practices should be followed throughout the year, especially at clean-up; monitoring when and where symptoms occur each year may help identify areas where more effective clean-up is required.
- The testing of young plants before transplanting (using the rapid qPCR test developed by Fera Science Ltd.) may help prevent the introduction of infection. Contact Fera Science Ltd. for information about this service.
- Reducing initial inoculum concentration of *R. radiobacter* resulted in slower development of tomato root mat. Treatments that suppress pathogen populations are likely to delay or prevent disease development. However, experience shows that established 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 reduced, or even eradicated – 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.
- The chlorine dioxide-based product Clorious2 can be used at full recommended rate without causing phytotoxic or corrosive effects during cropping. This provides significantly better control of *R. radiobacter* than hydrogen peroxide-based products such as Geosil and EndoSan3 when used against a catalase positive strain of *R. radiobacter.*
- The new formulation of Domestos (Domestos Extended Power, a.i. sodium hypochlorite) has been shown to effectively eliminate viable *R. radiobacter* biofilms in irrigation tubing in laboratory settings. Domestos Extended Power has a high active chlorine concentration, which is phytotoxic, and is therefore only recommended for the disinfection of surfaces at times such as end of season clean-up.
- Test plants at arrival to develop crop management strategies in situations where plants may arrive infected.
- Test water sources and flush the irrigation system routinely prior to plant arrival to lower inoculum and slow down symptom progression.
- Monitor hot spots year-on-year and consider additional treatment operations, e.g. replacing irrigation piping drip pegs, etc.

SCIENCE SECTION

Introduction

Root mat disease of tomato affects around 90% of UK tomato nurseries and is now a disease of global significance. Root mat was first confirmed in tomato in the UK in 2000 and observed in plants propagated in the Netherlands during 1999. The disease is caused by the bacterium *Rhizobium radiobacter* (previously *Agrobacterium* bv. 1) containing a root inducing *Rhizobium* cucumopine (Ri) plasmid. Part of this plasmid, the T-DNA, is transferred from the bacterium to a plant cell during root infection. The T-DNA contains genes, which are subsequently incorporated into the plant cells genome and expressed by the host cellular machinery. This results in the production of cucumopines, a food source for the bacterium, and a hormone imbalance, resulting in excessive, abnormal root proliferation, leading to the characteristic root mat symptoms.

Further investigation has revealed the potential for the Ri plasmid to be acquired by a number of other common soil borne bacteria, including members of the genera *Ochrobactrum*, *Rhizobium* and *Sinorhizobium,* which could act as a natural reservoir of the Ri plasmid for further development of root mat disease in both tomato and cucumber (Weller *et al.,* 2000). Crown gall, a related disease, is caused by tumour-inducing plasmids (pTi) in *Rhizobium* species. These bacteria containing the pTi plasmid have been associated with crown gall in raspberry (Khmel *et al*., 1998).

The most common and recognisable symptom of root mat disease in tomato is extensive root proliferation within, and on the surface of the propagation cube. Symptoms will usually spread across the slab surface as symptoms develop over time. Roots grow up and along the surface of the propagation cube, usually around the irrigation peg. In some examples, the roots may grow up the irrigation peg leading to blockages (Figure 2) and irrigation issues. Excessive root growth may in extreme cases lead to swelling and distortion within the cube (Figure 3) and the slab.

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 from non-pathogenic isolates which lack the root inducing plasmids. Non-pathogenic strains of *R. radiobacter* are ubiquitous in soils, circulating in liquid nutrient media and associated plant material.

Figure 2. Roots growing up the irrigation pegs

Figure 3. Swollen cube due to severe infection

A rapid molecular test validated by Fera Science Ltd. as part of this project, is now available and allows detection of T-DNA from cucumopine Ri plasmids in tomato roots prior to symptom occurrence. This permits accurate evaluation of infection (including pre-symptomatic infection) and strengthens the reliability of results from work investigating efficacy of control measures. The rapid nature of this test (standard turnaround time of 10 working days from receipt of sample) allows for quick determination of when infection occurs during plant growth and has been used to detect infection in young asymptomatic plant tissue at plant arrival on commercial sites during 2017 and 2018.

Growers questioned in 2015 reported the proportion of tomato crops affected by root mat disease was between 1 - 5%. The trial work in 2017 revealed the proportion infected on the two sites used was 4 - 35%, with an incidence of 54% in plants grown in once-used bags with a history of the disease by the final assessment. The disease was not as severe in the three 2018 trials, but was severe in other areas at two of the three host nurseries during this time. In 2016 no grower reported slight symptoms, with all recording symptoms as either moderate or severe (10% or above cube coverage). In 2018 many growers have stated that root mat disease incidence is increasing year-on-year and becoming a much greater concern. The first occurrences of plant death attributed directly to the disease on two sites was recorded during this project, although the disease had killed plants on other sites before this time.

Two growers who sourced their plant material from propagators in the UK, grew crops that showed no symptoms of root mat disease in 2017. UK propagators are restricted in the quantity and range of products they can apply at this stage and may have more stringent hygiene practices as a result. If propagation material arrives uninfected and site clean-up at

turnaround is comprehensive, it is entirely possible that growers could eliminate the disease from their sites and this has been reported by some growers.

Over two-thirds of growers questioned removed the plastic wrapper from slabs in order to reduce the impact of root mat disease and the efficacy of this strategy was tested as part of additional work in this project. The use of biological control products and managed irrigation in root mat disease management is less popular with growers, with the efficacy of these control measures varying greatly.

Growers consider the impacts of irrigation, subsequent drainage and aeration important. There is suggestion that light levels may play a role in symptom expression. It is believed by some growers that some varieties of scion, or rootstock / scion combinations were more susceptible. One variety, Kanavaro, has been observed to be less susceptible to root mat than others are (Van Kerckhove, 2015). Rootstock providers were contacted during 2018 to determine if any work was being undertaken to develop resistance to root mat disease in rootstocks. Of those questioned none were currently working on this as a priority. Tomato variety breeders were also approached with the same response.

Work performed in 2017 quantified the effect of two biological based products applied during propagation, at planting, in crop and at a combination of these timings, on infection and transformation of roots prior to symptom occurrence. This utilised the qPCR molecular test developed by Fera Science Ltd. to confirm infection, including times at plant arrival where plants may appear symptomless. Laboratory work performed by Fera Science Ltd. also determined the most effective biocides to use at crop turnaround on pure cultures and biofilms of *R. radiobacter.*

The results from 2017 showed that Carbon Gold treatment provided a positive reduction in both root mat disease incidence and severity in two of the three trials. The optimal timing and number of applications of Carbon Gold was inconclusive. The two 2018 efficacy trials aimed to further explore the use of Carbon Gold for control of tomato root mat.

Materials and methods

Objective 4

The two commercial sites which hosted the three root mat trials in 2017 hosted two further trials during 2018. Trial D, located in the South of England, was placed in the same area used for Trial A (2017), whilst Trial E (located in the East of England) was placed in a neighbouring compartment to that used for Trial B (2017). A third trial, Trial F, was established at a new site (Site 3), also located in the south of England. All trial areas on each site were selected

based on their history of root mat infection in the past. This maximised the likelihood for root mat symptoms to develop to sufficient levels for differences to be observed.

The use of Carbon Gold in 2017 successfully reduced the incidence and severity of root mat disease in both trials at Site 2. In these trials the number and timings of the applications of Carbon Gold needed to give the best control were conflicting. No Carbon Gold treatment was applied solely at planting alone in 2017, always incorporated with a treatment at the propagation stage. A Carbon Gold treatment at planting alone was included in the 2018 work in Trial D to determine the ability of Carbon Gold to manage the disease at this time alone.

Three Vitix treatments were tested in 2017 but were not carried forwards to the 2018 trials due to the negative results seen in 2017. However, a combined Vitix and Carbon Gold treatment at propagation was trialled. This was chosen as a small, but significant reduction in root mat severity was observed at one assessment during mid-cropping in 2017. This was included to determine if there was a combined effect of the two treatments. The rate of Vitix application was reduced from the 1.5 g per m^2 used in 2017 to 0.15 g per m^2 following advice from the manufacturer Koppert.

Because of the changes in growing practices between the sites, and the limited availability of product, exact replication of the three trials at all three sites was not possible. Treatment number varied between trial, but in all cases the trials included a double untreated control in a fully randomised blocked structure with six replicates. For statistical analyses, the 12 untreated plots were treated as one treatment in order to give a single mean value.

All statistical analysis was performed by the ADAS statistician Chris Dyer who was consulted with regard to initial experimental design to ensure the results gained in this project were scientifically robust. Analysis of variance (ANOVA) of the incidence and severity of root mat disease was performed where statistical analysis was appropriate. Information for each trial is included in Table 1.

Table 1. Information for the three trials including, variety, rootstock, slab and cube choice, the number of cubes per plot and propagator - 2018

Site	Trial	Propagator	Variety	Rootstock	Slab type	Cube type	Cubes /plot
1	D	A (British)	Piccolo	Optifort	Botanicoir DRY	Botanicoir	84
2	E	B (Dutch)	Piccolo	Optifort	Cultilene Exact Air Gradient	Plantop	80
3	F	A (British)	Sunstream	Optifort	Grotop master	Plantop	96

All trials used brand new slabs containing two individual cubes with one plant planted per cube. Plants for trials D and F were grown in separate areas at the propagator

Trial D

Piccolo plants were sown on the 7th November 2017. This UK based propagator applied the Carbon Gold and Vitix treatments on behalf of the project. Tomato plug plants were treated with product following manufacturer recommendations (Table 2) with a large quantity of plants (around 3,500) treated. During propagation, each block of treated and untreated plants were kept separate. Conventional fungicide use was also minimised, only used if absolutely necessary. Vitix was applied as a hand drench and Carbon Gold was hand dusted around the plug. Seed samples and an ebb-flood solution sample were also requested from the last watering to be tested by Fera Science Ltd. for the presence of the Ri plasmid.

At planting, December 28th 2017, 55g (approx. 1 scoop) of Carbon Gold was placed between the slab and cube for treatments 5 and 6 by nursery staff. The slabs used were Botanicoir DRY (product code: BR01_1001511_W) and cubes used were Botanicoir (product code: GC01_101006_NW).

Table 2: A summary of treatment timings and doses of the treatments used in Trial D at both propagation and at planting. Site 1, November 2017 – January 2018.

Treatment	Product	a.i.	Rate	Timing	Responsibility
1	Untreated	-	-	-	Nursery staff
2	Untreated	-	-	-	Nursery staff
3	Carbon Gold	Biochar + biology blend	5g at propagation	In propagation	Propagator
4	Carbon Gold	Biochar + biology blend	55g at planting	At planting	Nursery staff
5	Carbon Gold	Biochar + biology blend	5g at propagation, 55g at planting	In propagation & at planting	Propagator and Nursey staff
6	Carbon Gold and Vitix (reduced rate)	Mixed inoculation	Vitix - 0.15g per m^2 at propagation Carbon Gold - 5g at propagation	In propagation only	Propagator

All treatments were applied by either the propagator and / or the host nursey staff.

Trial E

Due to changes to the growing practices at Site 2, no propagation treatments were included in Trial E. Piccolo plants sown on 4th December 2017 were planted out onto slabs on the 2nd February 2018. Only one treatment of Carbon Gold, applied at planting alone, was included (Table 3).

The rockwool slabs used were Cutilene Exact Air Gradient and the rockwool cubes Plantop Delta.

Table 3. Summary of the timings and doses of treatments in Trial E, Site 2- 2018

Treatment	Product	a.i.	Rate	Timing	Responsibility
1	Untreated	-	-	-	Nursery staff
2	Untreated	-	-	-	Nursery staff
3	Carbon Gold	Biochar + biology blend	55g between the cube and the slab	At planting	Nursery staff

All trials used brand new slabs containing two individual cubes with one plant planted per cube

Additional swabbing – Trial E

During the 2017 trials qPCR testing was performed by Fera Science Ltd. on composite root samples collected at plant arrival for each trial. In all cases some roots tested positive for root mat disease infection, indicating this occurred at the propagation stage. It is not possible to be 100% certain that infection did occur at this stage, as infection may have arisen during the transportation of the plants to the host nurseries.

To investigate this, equipment involved in the transportation of plants to Site 2, the delivery lorry and trolleys, were swabbed to identify whether *R. radiobacter* containing the Ri plasmid was present and if this could be a potential route for infection to occur following departure from the propagator.

Site 2 suffered from very severe root mat infection during 2017, including the compartment where Trial E was placed. Areas of the site infrastructure within the trial area were also swabbed at plant arrival to confirm the presence / absence of *R. radiobacter* on Site 2. The locations of all the areas swabbed are located in Table 4.

Table 4. The location of the swabs taken from Site 2, including from the delivery lorry, trolleys and site infrastructure, to determine presence of *R. radiobacter* with the Ri plasmid pre-planting – February 2018

Swab number	Location
1	Lorry (left wall, front)
2	Lorry (left wall, back)
3	Lorry (Left back corner, near the wall)
4	Lorry (floor, left hand side at back)
5	Lorry (back wall)
6	Lorry (floor by back wall)
7	Lorry (floor, front)
8	Lorry (tail lift floor)
9	Trial plant trolley 1
10	Trial plant trolley 2
11	Leaking irrigation valve (valve 29), in trial area
12	Site 2 trolley
13	Gap in the concrete pathways between plots 6 and 15
14	Pooled (greening) water on new polythene (plot 15, cube 46)
15	Exposed soil of glasshouse (plot 10, cubes 2 & 3)

Images of the locations for each of the swabs are located in Appendix 1.

Trial F

Trial F, cv. Sunstream, did not involve either Carbon Gold or Vitix treatments. This trial further investigated complementary work to this project performed on outdoor cucumber by Village Farms (USA) during 2016. Many growers believe that the removal of the plastic wrapper from the surface of slabs may reduce root mat symptom severity by aiding aeration and light interception. The plastic on the surface of the rockwool slabs and / or the plastic sleeve surrounding the rockwool cubes in Trial F was removed on some rows to determine the effectiveness of this process in controlling root mat incidence and severity (Table 5). Site 1 also removed the plastic surface from the tops of their slabs in Trial D (as part of their standard growing practices), whilst Site 2 chose to retain the plastic surface in Trial E. Neither sites removed the sleeves surrounding the cubes.

Table 5. Treatment list for Trial F - plastic removal trial, Site 3 - 2018

Treatment number	Level of plastic removal
1	None (untreated)
2	None (untreated)
3	Cube sleeve
4	Cube sleeve and slab surface

All plastic removal (slabs and cubes) was performed just prior to planting (Jan 2018)

The plastic was removed at planting (12th of January 2018) by nursery staff leaving a 3 cm border around the surface of the slab (Figure 4). The slabs used were Grotop Master and the cubes Plantop.

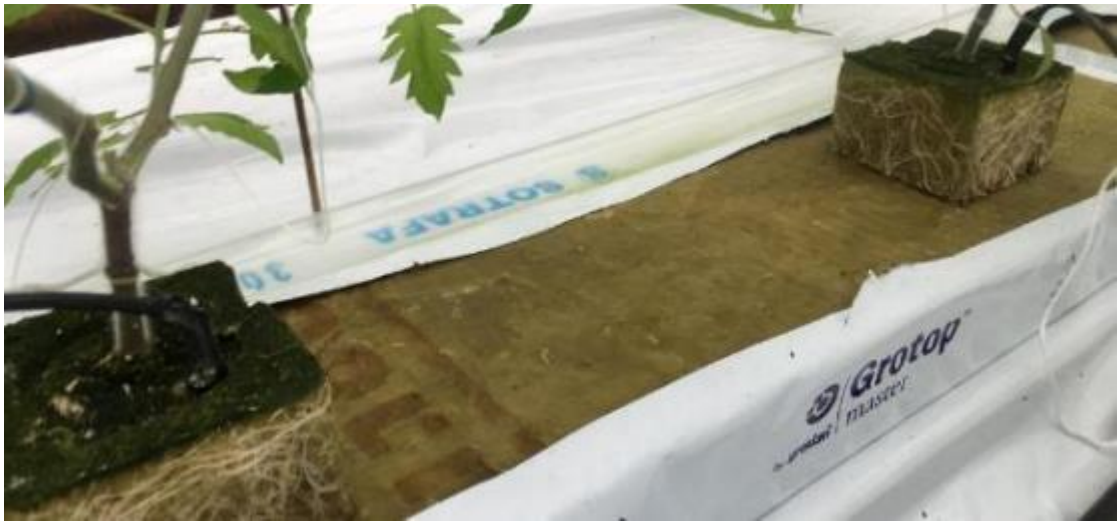

Figure 4. Example of a plot with the plastic surface of the slab and cube sleeve removed

Assessments

Trials D-F were visually assessed four times throughout the year, at plant arrival, first symptom development, mid-cropping and at the end-of-cropping. All cubes in each plot were assessed for visible root mat disease incidence and severity at each assessment. At plant arrival a preliminary visual assessment of plant vigour, and the occurrence of tomato root mat (number of plants infected) was recorded. The dates for each assessment are located in Table 6.

Table 6. The dates of the first-symptom, mid-crop and end-of-cropping assessments in Trials D, E and F during 2018

	Date		
Assessment	Trial D	Trial E	Trial F
First-symptom	04/07/18	27/06/18	11/04/18
Mid-crop	31/08/18	30/08/18	17/07/18
End-of cropping	26/10/18	25/10/18	25/10/18

All cubes were assessed for the incidence of root mat disease and the severity based on the scoring criteria in Table 7.

Table 7. Severity scores based quantity of root mat coverage on the propagation cube

Score	Severity
0	No root mat symptoms
1	1 or 2 upright roots on the cube
2	5% of cube surface affected
3	6 - 10% of the cube surface affected
4	11 - 20% of the cube surface affected
5	21 - 50% of the cube surface affected
6	Greater than 50% of the cube surface affected

The location of each infected cube per plot relative to other plants was also recorded at these in-crop assessments to aid in the development of “heat maps” for each trial. The ‘heat maps’ provide a visual representation of the distribution and severity of the disease over the duration of the trial period which may provide insights in understanding the epidemiology of the disease in these commercial settings and are located in Appendix 2

Root sampling for testing at Fera Science Ltd.

At the first-symptom and end-of-cropping assessments, composite root samples were collected from all treatments in Trials D, E and F. These samples were sent to Fera Science Ltd. for the detection of the Ri plasmid T-DNA using the rapid qPCR test validated in Objective

2. These root samples were tested in addition to the seed and water samples sent from the propagator used by Sites 1 and 3. On all sampling occasions, nitrile gloves were used and disinfected or changed between sampling different treatments to prevent cross-contamination.

To determine if asymptomatic young plants were infected at arrival on site, a small pinch of roots (1-2 cm) was taken from 200 plants from the four propagation treatments, untreated, Carbon Gold and Vitix / Carbon Gold (Trial D). Samples from each treatment were collected and placed into four separate bags for testing. Root samples were also collected at arrival and sent for qPCR analysis following the same sampling procedure from each treatment in Trials E and F. As no propagation treatments were performed in these trials a subsample of roots were collected across all plants in each trial at arrival, before planting.

At the first-symptom assessment, root samples from ten symptomatic plants and ten asymptomatic plants were collected from the propagation cubes. In cases where ten symptomatic plants were not present, all symptomatic plants in each plot were sampled. In cases where no infection was present no symptomatic root samples could be collected. Root samples were bulked together to produce two bags per plot, one containing symptomatic and the other asymptomatic roots. The root sampling procedure was repeated at the end-of-cropping assessment to determine if root mat disease infection was now present in previously uninfected plots.

All three crops were grown to commercial standards with host growers requested not to apply other biological products which may interact with the Carbon Gold and / or Vitix treatments unless necessary, however Site 2 did dose with the NatuGro program throughout the season. During 2018, Site 1 dosed their irrigation system with Chlorious2, the chlorine dioxide product which gave the best control in the biocide testing performed by Fera (Objective 5).

Shortly after the final assessment two irrigation solution samples (2x 50ml) were collected from drippers and sent to Fera to test for the presence of *R. radiobacter* within the system. Three drip pegs were also collected from cubes exhibiting severe infections from each site to determine if *R. radiobacter* could be isolated from these.

Results

Objective 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;

Work under this objective also further examined the effect of treatments applied in propagation (Objective 3)

Testing of seed and water samples collected at propagation

Plants for Trials D and F were sourced from the same UK propagator. Samples of the seed batches used in Trial D (Piccolo) and Trial F (Sunstream) were sent directly from the propagators to Fera Science Ltd. with all testing negative for *R. radiobacter* and the Ri plasmid. This result suggests that the bacterial cause of root mat disease is not present on / in commercial tomato seed and this corresponds with the seed results from 2017. Water samples taken at the final irrigation event for the three propagation treatments used in Trial D (Carbon Gold, Carbon Gold and Vitix and Untreated) also tested negative for *R. radiobacter* and pRi. The absence of the Ri plasmid in the water samples suggests the propagator used, and the areas used to store the tomato plants for both sites, were free of root mat disease. It should be kept in mind that the seed samples (~100 seeds) were relatively small and that the water samples represented only a single point in time and the possibility of seed and / or water-borne infection cannot be completely discounted. No water samples were provided for testing from the first irrigation event from either of the propagators and no seed or water samples were gathered from the Dutch propagator supplying Trial E.

Testing of composite treatment samples at plant arrival

Trial D

For each of the three treatments applied at propagation in Trial D, a small sample of untreated, Carbon Gold, and Carbon Gold and Vitix treated plant roots were tested. No infection was found in the root samples collected from the untreated or combined Carbon Gold and Vitix treated populations (Table 8). The presence of pRi DNA was detected via direct DNA extraction in samples taken from the Carbon Gold roots (CT score of 35.85), but was not detected before and after incubation in an enrichment medium. The direct test was repeated and gave a negative result for Ri plasmid presence. These results suggest that infection was present in the plants treated with Carbon Gold, but not all the plants were infected. A subsample of the 200 composite root sample collected was tested and it is possible that the first direct extraction included roots which contained infected tissue and the repeat did not. It is also possible that the overall level of infection was at the limit of detection for the assay, but is unlikely as a low / average level of infection was detected.

Table 8. qPCR results from composite root samples collected pre-planting from the three propagation treatments used in Trial D, Site 1 - January 2018

Treatment	Pre-enrichment (Ri CT)	Post-enrichment (Ri CT)	Direct (Ri CT)	Direct repeat (Ri CT)
Untreated	40.00	40.00	40.00	-
Carbon Gold	40.00	40.00	**35.85**	40.00
Carbon Gold + Vitix	40.00	40.00	40.00	-

The value in bold indicates detection of the Ri plasmid at a low / average level of infection.

Direct extraction Cox CT (control) – 19.70 Negative result Cox CT - 40.00

Cycle threshold (CT) values indicate the number of PCR cycles for a fluorescent signal to exceed a certain threshold level. A high CT score indicates a low average level of infection, and a CT score of 40 represents a negative result, with no target amplification over 40 PCR cycles. A CT score of 37 indicates the presence of 1000 – 10,000 pRi plasmid-containing cells per ml of extract and a CT difference of 3 roughly equates to a 10-fold difference in populations. An internal positive control (Cox), which amplifies cytochrome oxidase gene sequence from the tomato tissues, was run alongside each test and CT values obtained indicated successful DNA extraction and amplification from each sample. The sampling rate per treatment is sufficient to detect an infection level of 1.5% or more with a confidence of 95%.

Trial E

Plants for Trial E were propagated in the Netherlands at the same large Dutch propagator which supplied the plants to Trials at Site 2 during 2017. This propagator is not GSPP certified, but does follow GSPP guidance. A composite sample of roots from plants destined for the untreated and those to be treated with Carbon Gold at planting were collected prior to planting. As plants received no propagation treatments the results were combined (Table 9). This shows that considerable levels of infection were found. Despite this the plants all appeared healthy with no visible symptoms of root mat disease (or other visible pathogen).

Table 9. Average qPCR results from composite root samples collected pre-planting from tomato plants used in Trial E, Site 3 – February 2018

Treatment	Pre-enrichment (Ri CT)	Post-enrichment (Ri CT)	Direct (Ri CT)
Untreated (all plants)	36.59	33.26	28.36

Direct extraction Cox CT (control) – 25.38 Negative result Cox CT - 40.00

Additional Swabbing

At the date of plant arrival on Site 2, areas of the delivery lorry, trollies and site were swabbed to test for the presence of *R. radiobacter* and the Ri plasmid. The lorry and trolleys were chosen to determine if infection could potentially have occurred on route from the propagator. The site was swabbed to establish if a viable source of inoculum was present on Site 2 at the time of plant arrival. The areas swabbed are listed in Table 4 and all tested negative for *R. radiobacter* and pRi. In addition to these swabs, two sets of irrigation water taken from drip pegs evenly distributed over the trial site (two each side of the central walkway) were collected and sent to Fera Science Ltd. Both tested negative for the bacteria pre and post enrichment (CT 40.00).

Trial F

No treatments were applied by the large UK based propagator which supplied the plants used in Trial F. Two hundred root samples were collected and sent to Fera Science Ltd. from what would become each of the plastic removal treatments (800 samples in total). The results for the composite root samples all returned negative (CT 40.00) suggesting the plants were free of root mat infection.

Trial D was supplied by the same propagator as Trial F and infection was detected in plants treated with Carbon Gold alone in Trial D. In total, roots from over a third of the plants in Trial F were sampled, increasing the confidence that the plants in this trial were free of root mat disease. The plants destined for trial D (cv. Piccolo) and those for trial F (Sunstream) were propagated in different locations. If *R. radiobacter* is present at propagation it is unlikely to be spread uniformly over propagator sites and this may explain the differences in infected plants delivered from one propagation site.

A summary of all qPCR testing conducted in 2018 is given in Table 10.

Table 10. qPCR results for the testing of seed, irrigation water and plant roots at propagation, plant arrival and the first-symptom and end-of-cropping assessments for presence of *R. radiobacter* and the Ri plasmid in the three 2018 commercial trials

Sample type	Detail	Date collected	Number of tests	Number positive for pRi plasmid
Trial D (cv. Piccolo) – Site 1				
1. Seed	Propagator	15/01/2018	2	0
2. Water	Propagator	23/12/2018	4	0
3. Water	Arrival at Site 1	28/12/2018	-	-
4. Water	End-of-crop	26/10/2018	2	0
5. Roots	Arrival at Site 1	28/12/2018	3	1
6. Roots	Asymptomatic	04/07/2018	36	4
7. Roots	Symptomatic	04/07/2018	4	1
8. Roots	Asymptomatic	26/10/2018	36	6
9. Roots	Symptomatic	26/10/2018	1	1
Trial E (cv. Piccolo) – Site 2				
1. Seed	Propagator	-	-	-
2. Water	Propagator	-	-	-
3. Water	Arrival at Site 2	02/02/2018	2	0
4. Water	End-of-crop	25/10/2018	2	1
5. Roots	Arrival at Site 2	02/02/2018	3	3
6. Roots	Asymptomatic	27/06/2018	18	18
7. Roots	Symptomatic	27/06/2018	3	3
8. Roots	Asymptomatic	25/10/2018	18	18
9. Roots	Symptomatic	25/10/2018	3	3
Trial F (cv. Sunstream) – Site 3				
1. Seed	Propagator	15/01/2018	2	0
2. Water	Propagator	23/12/2018	4	0
3. Water	Arrival at Site 3	12/01/2018	2	0
4. Water	End-of-crop	25/10/2018	2	0
5. Roots	Arrival at Site 3	12/01/2018	4	1
6. Roots	Asymptomatic	11/04/2018	24	10
7. Roots	Symptomatic	11/04/2018	3	3
8. Roots	Asymptomatic	25/10/2018	24	22
9. Roots	Symptomatic	25/10/2018	14	14

- No seed and / or water samples were sampled.

Preliminary assessments – at plant arrival

Root mat incidence and plant vigour was visually assessed for all trials and no symptoms of root mat disease were observed. Plant vigour was assessed by the staff at Site 1 in January and the plants were scored as visibly uninfected and healthy, with a medium vigour score of three (1 = very poor, 5 = perfect) in Trial A. Highly vigorous plants are not desirable at plant

arrival as strong plants are more susceptible to botrytis infection. Plants at Site 2 and 3 were also assessed at plat arrival, with both scoring three. Scoring of the plants in these trials were performed by ADAS.

In crop assessments

Trial D

First symptoms - Plants arrived at Site 1 on the 28th of December 2017 with the first root mat symptoms detected on this site on January 16th 2018 (the first incidence of root mat disease did not occur within the trial area). No root mat infection was reported within Trial D by staff at Site 1 and the trial was visited on July 4th 2018 to collect root samples to check for asymptomatic infection. Only one incidence of the disease was observed in plot 35 (cube 60), a cube treated with Carbon Gold at both propagation and at planting. This single cube was severely infected with root mat symptoms progressing to the slab (Figure 5). The neighbouring cube on the same slab showed no infection.

Figure 5. The severely infected cube in Trial D

Figure 6. The neighbouring cube with no infection

Root samples from 10 asymptomatic plants / plot were collected and sent to Fera Science Ltd. for qPCR analysis, alongside root samples from the single infected cube. Despite only

the one visually symptomatic cube present in the trial, five plots were found to contain infected plants (Table 11).

Although the number of infected plots was low, the majority of root mat infected plots (four of the five) were plots treated with Carbon Gold (without the addition of Vitix). This is an anecdotal observation and greater levels of disease incidence would be required to determine this statistically. Interestingly the ten samples collected from asymptomatic plants from plot 35, containing the severely infected cube, did not test positive for root mat infection. The infected cube did test positive, with a direct extraction CT score of 20.47, a very severe infection. Full qPCR results from the first-symptom and end-of-cropping assessments for Trials D, E and F are located in Appendix 3.

Table 11. The number of infected plots and average CT score per treatment in Trial D at the first symptom assessment, Site 1 - July 2018

Treatment	Timing	No. plots / treatment	No. infected plots / treatment	Average direct Ri CT score / treatment
Untreated	-	12	1	39.9
Carbon Gold	Propagation	6	0	40.0
Carbon Gold	Planting	6	0	40.0
Carbon Gold	Propagation & at planting	6	3	34.7
Carbon Gold & Vitix (reduced rate)	Propagation	6	1	38.8

Mid-cropping - Due to the delay of the first-symptom development in Trials D and E the timings of the mid-crop assessments were scheduled to take place midway between the first-symptom and the end-of cropping assessments (late October / Early November), rather than the middle of the cropping season (July). This avoided any two assessments from taking place in rapid succession of one another which would be unlikely to yield much in the way of differences.

The mid-crop assessment at Trial D took place on the 31st of August, 60 days after the first symptom assessment. No further cases had developed in any cubes at this time with just plot 35 (cube 60 alone) continuing to show severe infection. The root matting on the surface and side of the cube and slab which was exposed to the glasshouse environment had discoloured and was dry / dead (Figure 7) compared to how it had appeared at the first-symptom assessment in on the 3rd of July.

Figure 7. (Left) Root mat symptoms in row 35 (cube 60) at the mid-crop assessment (31st August 2018) with dead / decaying root mat symptoms on the slab surface

Figure 8 (Right). Root mat symptoms in row 35 (cube 60) at the end-of-cropping assessment (26th October 2018)

The low level of visually symptomatic plants in Trial D was unexpected, especially as more plots had been demonstrated to contain infected plants. Roots sampled at the first symptom assessment, from plants treated with Carbon Gold alone, gave a CT score of 34.7, a moderate level of infection and visual symptoms would be expected to develop.

End-of-cropping – Site 1 was visited on the 26th October 2018, 57 days following the mid-crop-assessment. Again no new visibly infected cubes had developed. In some cases, where the plastic surrounding the sides of the slabs was pulled free, root mat symptoms were visible. Despite this, symptoms had not developed onto the surface of the cube, or the exposed surface of the slab. The single infected cube in this trial continued to show greater than 50% cube coverage, however the overall coverage had not visibly increased since the first-symptom assessment in April (Figure 8).

Root samples were collected for qPCR analysis to determine if infection had spread to more than the five plots infected in April. Water samples and drip pegs were also sampled for testing for *R. radiobacter* and pRi presence.

Both water samples tested negative for *R. radiobacter* and pRi presence, the same result was achieved from the water samples tested at plant arrival in January implying the irrigation

system is not the source of disease inoculum. Testing of the drip pegs did reveal presence of the bacteria (CT 26.1) post enrichment.

Sampling of roots showed six plots were infected in November, an increase from five in July. Some plots which had tested positive for infection in July however no longer tested positive for infection in November. Each plot in Trial D comprised 84 tomato cubes and only ten random root samples from asymptomatic plants were collected. As a consequence of very low disease incidence in Trial D, sporadic infected plants in each plot may have been sampled in July, but not in November.

The qPCR results from the end-of-cropping sampling and a combination of both the July and November sampling dates are shown in Table 12 giving a total number of infected plots per treatment for the season.

Table 12. The number of plots confirmed infected with *R. radiobacter* (via qPCR) per treatment at the end-of-cropping assessment (Nov 2018) and combined values for both first-symptom and end-of cropping assessments for trial D.

Treatment	Timing	No. plots / treatment	No. infected plots / treatment at end-of-cropping assessment	No. infected plots / treatment (both assessments combined)
Untreated	-	12	1	2
Carbon Gold	Propagation	6	2	2
Carbon Gold	Planting	6	1	1
Carbon Gold	Propagation & at planting	6	2	3
Carbon Gold & Vitix (reduced rate)	Propagation	6	0	1

The combined results for both qPCR assays shows that no treatment was able to prevent root mat symptom developing.

Due to the single infected plant in Trial D at all assessment dates it was not possible to perform any meaningful statistical analysis.

Trial E

First symptoms – The Piccolo crop was planted onto slabs on February 2nd 2018. These plants were known to be infected following qPCR testing at arrival (Table 9). Symptoms were

first observed on Site 2 on May 10th 2018, but not within the trial area. The trial was visually assessed on June 15th and no symptoms were found. The trial was visited again on June 27th 2018 with one confirmed infected cube recorded. The visibly infected cube was located in plot 11, cube 70, in an untreated plot. This cube was severely infected with over 50% of the cube surface covered with root mat symptoms. Swelling of the cube in the corner of the plastic sleeve where the irrigation peg enters the cube can be clearly seen (Figure 9).

Figure 9. The severely infected cube at the first symptom assessment - June 2018

Ten root samples from plants asymptomatic of root mat infection per plot were bulked together, as well as roots from the infected cube, and sent to Fera Science Ltd. for qPCR analysis. Every individual plot was shown to contain infected plants asymptomatic of root mat disease (Table 13). This was expected as earlier qPCR analysis results had shown that plants arrived infected. What was unexpected was the lack of visual disease development considering the extent of infection likely present within the plants.

Table 13. The number of infected plots and average CT score per treatment in Trial E at the first-symptom assessment – Site 2, February 2018

Treatment	No. infected plots / treatment	Average direct Ri CT score / treatment
Untreated (12 plots)	12	26.6
Carbon Gold at planting (6 plots)	6	27.5
Direct extraction Cox CT (control) – 19.70	Negative result Cox CT - 40.00	

Mid-cropping – Trial E was assessed on August 30th 2018, 64 days following the first symptom assessment. Root mat disease incidence increased only slightly with a total of four cubes visually infected at this date (three untreated and one treated with Carbon Gold at planting). The severity of root mat symptoms was varied with only one cube showing greater than 50% cube surface coverage at this time

The average CT score of 27.5 from roots sampled from Trial E was found at the first-symptom-assessment across all plots. This is a significant level of root infection, considerably more than the equivalent scores seen in Trials D and F and is a level at which visible infection would be expected to be seen.

End-of-cropping – The end-of-cropping assessment was carried out on the 25th October 2018, 57 days after the mid-crop-assessment. Only one additional plant developed root mat disease during this time, with just five in total within the trial area. Of these five, four cubes showed severe infections between 21 and 50% cube coverage. Infection across the entire site was extremely low, despite the high levels of infection seen during 2017 and the fact that the plants arrived infected with root mat disease.

Roots were collected and retested using the qPCR assay and infection was confirmed in all plots across untreated and Carbon Gold treated plants except for plot 14, Carbon Gold. This plot was shown to be infected at the first symptom assessment and was due to insufficient sample size as described in Trial D above. Full qPCR results are located in Appendix 3.

Two water samples were collected at the end-of-cropping assessment. One of these samples tested positive for *R. radiobacter* showing a significant bacterial presence (CT 30.97). Drip pegs tested also tested positive (CT 30.7). An additional irrigation lace was also sent for analysis and *R. radiobacter* was isolated from this (CT 20.67). This suggests that there are biofilms of *R. radiobacter* within the irrigation system and it may have a role to play in infection at Site 2.

Due to the low number of infected plants in Trial E at all assessment dates it was not possible to perform any meaningful statistical analysis.

Trial F

First-symptoms – Tomato plants cv. Sunstream were planted on Site 3 on January 12th 2018 immediately after removal of the plastic surrounding the cube sleeve and slab where required by the treatment plan. Root mat disease was spotted at this site on March 14th and within Trial C on April 5th. The trial was visited on April 11th for a full visual disease assessment, and to collect root samples. qPCR analysis revealed that the plants within Trial F arrived free of root mat disease infection and no visual infection was seen in the first 19 (of 24) plots. Only

two plots, plot 21 (untreated) and plot 24 (cube sleeve and slab surface plastic removal), showed visual infection. Ten of the cubes in plot 21 were infected, with five cubes exhibiting 21-50% of slab surface coverage. Five more cubes showed greater than 50% cube coverage (Figure 10). The two infected cubes in plot 24 also showed greater than 50% cube coverage (Figure 11).

Due to extremely low levels of visible infection no statistical analysis was performed.

Figure 10. Severely infected untreated cube (no plastic removal)

Figure 11. Severely infected cube with sleeve and the surface of the slab removed

Results from the qPCR testing of root samples sent to Fera Science Ltd. for analysis, revealed that 14 of the 24 plots contained infection (Table 14). Plots 21 and 24 which contained the visually infected cubes were included in the plots with confrimed infection at this time.

Table 14. The number of infected plots and average CT score per treatment in Trial F at the first-symptom assessment, Site 3 - April 2018

Treatment	No. infected plots / treatment	Average direct Ri CT score / treatment
Untreated (12 plots)	4	38.5
Cube sleeve removal (6 plots)	1	39.7
Cube sleeve and slab surface plastic removal (6 plots)	5	38.4

Negative result Cox CT - 40.00

Interestingly the average direct Ri CT score at arrival in Trial E was 28.36 (Table 9), but at the first-symptom assessment the CT result was higher at 38.9.

Insufficient data was available for meaningful statistical analysis to be performed following the first-symptom assessment of Trial F.

Mid-cropping – As the disease occurred much earlier in Trial F, a greater time period was possible between the first-symptom and mid-crop-assessments (98 days). Site 3 was visited on July 17th 2018 and the total incidence of root mat disease across the entire trial had increased from 10 to 24 visually infected cubes. Although this did represent an increase in incidence, the total number of affected cubes remained low. At the mid-crop assessment the incidence was spread more uniformly over the trial, with half of the plots showing at least one infected cube / plot. Despite this spread, 78% of infected cubes were located in plots 21-24, with 42% of these infected cubes in plot 21. Generally the spread of infection mirrored the plots which were asymptomatic, but had infection confirmed by qPCR analysis at the first-symptom assessment. Plots 18, 19, 22 and 23 were not shown to be infected by the qPCR analysis at this time, but did develop root mat symptoms by the mid-crop-assessment. This suggests that the quantity of roots sampled was insufficient to accurately determine if plots were infected, or that the level of infection was too low to be detected at the sampling date, or that infection occurred after the first sample occasion.

Statistical analysis revealed no significant differences in disease incidence (Figure 12, P=0.622) or severity (P=0.577) between any treatments and the untreated control. Unsurprisingly statistically significant differences were seen in incidence (P=0.006) and severity (P=0.013) between blocks indicating location in the glasshouse played a role in disease incidence and symptom expression. This is further discussed in the additional work section in the discussion.

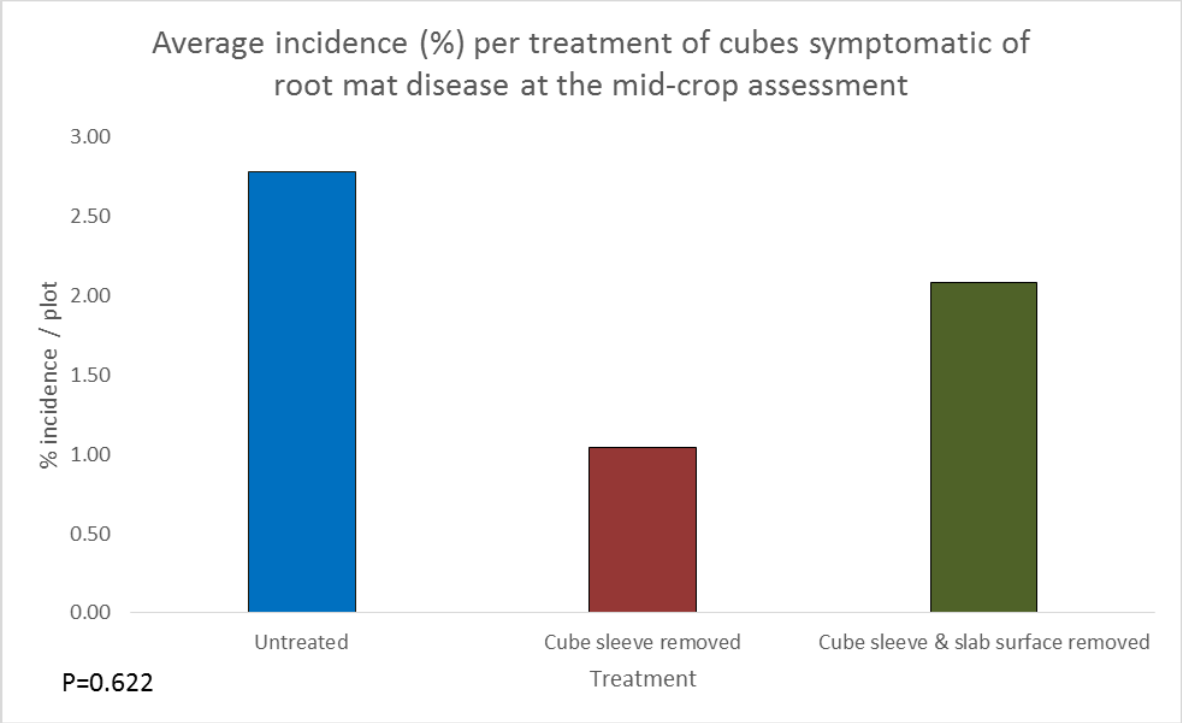

Figure 12. Incidence (%) of root mat disease in treated and untreated plots in Trial F at the mid-cropping assessment, July 17th 2018.

End-of-cropping – The plastic removal trial was visited for the final assessment on October 25th 2018, 100 days following the mid-crop-assessment. At this date 14 plots were visibly infected (up from 12). Despite an increase in total incidence from 50 to 85 affected cubes across the trial, the overall relative numbers of infected cubes remained low (infected cubes represented 3.7% of total cubes within the trial). Plot 21 continued to show extremely high numbers containing 45% of all the visibly infected cubes in the trial. At this date the cubes which were infected displayed severe infection with over 90% exhibiting greater than 10% cube coverage with root mat symptoms and 68% showing greater than 50% coverage.

The very high levels of root mat disease which occurred in plot 21 was considered an outlier and not representative of the overall pattern of distribution of root mat disease within Trial F. As a consequence this data was excluded from the statistical analysis.

Analysis revealed no significant differences in disease incidence (P=0.556) or severity (P=0.554) between any treatments and the untreated control.

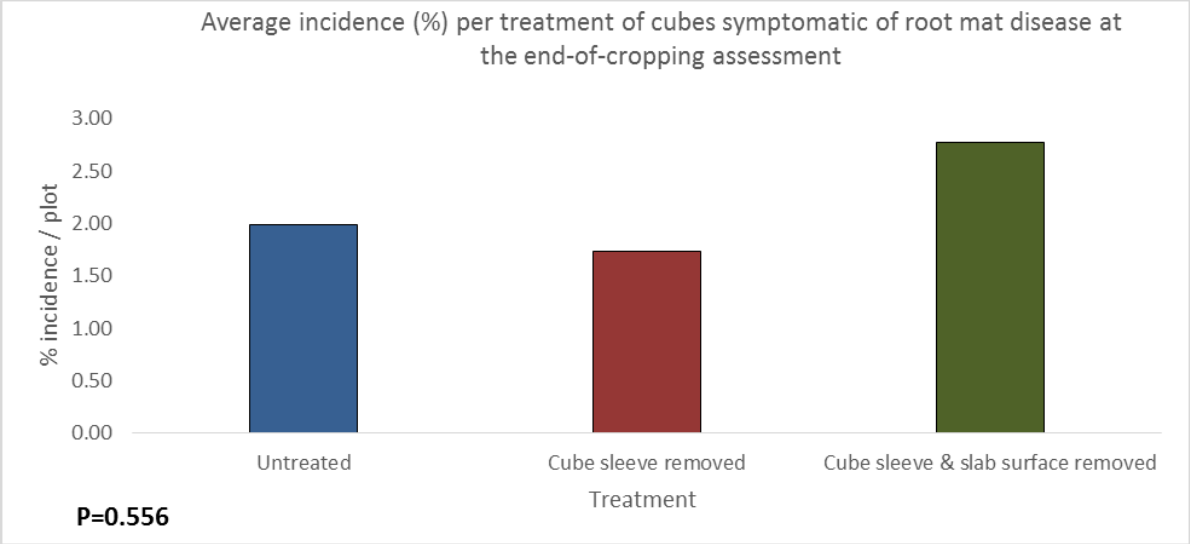

Figure 13. Incidence (%) of root mat disease in treated and untreated plots in Trial F at the end-of-cropping assessment, October 25th 2018. Data from plot 21 that had very high levels of root mat disease were excluded from the analysis as this plot was considered not representative of the overall pattern of distribution of root mat disease within Trial F.

qPCR testing of composite root samples at the first-symptom assessment in April revealed that although visible infection was only present in two of the 24 plots, 12 plots contained plants with transformed roots. At the final assessment this number had increased to 22 plots with just plots 3 (untreated) and 12 (cube sleeve removed).

The two water samples collected at this time tested negative for *R. radiobacter*, however the drip pegs (CT 20.74) and irrigation tubing tested positive (CT 19.72).

Discussion

Despite the strategic placement of the three trials in areas of historically high root mat disease pressure, very low levels of disease developed during 2018. As a consequence of this, no meaningful statistical analysis was able to be performed in either of the product efficacy trials D and E.

Although these two trials were unsuccessful it is important to consider why such low levels of infection occurred in the trial areas and what can be learned from this. Following on from the positive results in Objective 5 (2017), Site 1 dosed Trial D with Clorious2. Due to a dosing mistake shortly after planting, an extremely high dose of Clorious2 was run through the irrigation lines onto the newly planted crop. The design of the irrigation set-up meant that around 70% of the crop receive this extreme dose. This had a negative short term impact, damaging the roots of affected plants. These plants recovered however, and there was no discernible differences in overall plant health, fruit yield or quality by the end of the year. One

consequence of this would be the sterilisation of the equipment in this portion of the trial area and no infection occurred in these plots throughout the season. The only incidence of root mat disease which did develop was in a plot which received the standard Clorious2 dose. This cube, and other non-trial areas which also developed symptoms in the same compartment, despite being correctly dosed, demonstrates that Clorious2 (at standard dose rate) was insufficient to completely control root mat disease, but may be effective in reducing it. As disease presence was so low it is impossible to claim that the accidental dose of Clorious2 was the reason for the absence of disease in most of the trial area, however this is possible. Dosing of Clorious2 at this rate as a control strategy would be prohibitively expensive and inadvisable.

Site 2 saw very severe infection across the site in 2017 (Trials B and C). It is generally very difficult to successfully eliminate any infection from one year to the next following a significant disease presence, especially for bacterial infections. As a consequence of this, much greater levels of infection were expected to occur in Trial E than did eventually develop. At arrival, plants were tested for the presence of *R. radiobacter,* pRi and transformed roots. The qPCR analysis revealed that roots were infected at this time. These plants were sourced from the same large Dutch propagator as in 2017 and showed greater levels of infection than the previous year. Despite this, the plants used in 2017 developed significantly greater levels of infection than in 2018, even when grown in new, unused coir slabs.

Contrary to practices in 2017, the grower at Site 2 chose to use the NatuGro program in the trial area. This program routinely introduces nutrients and microorganisms, including *Trichoderma harzianum* T-22, to create a microbiologically enhanced root zone environment. The program claims to improve overall plant health and may suppress pathogenic fungi and bacteria. There is currently no direct evidence for the efficacy of this product against *R. radiobacter*, but it may have played a role. NatuGro was used across the entirety of Site 2 and further work into this warrants attention.

At the end-of-cropping assessment, irrigation solution, drip pegs and irrigation laces were tested for *R. radiobacter* presence and the bacteria was found to be present in all three of these, prior to any chemical treatment. This is a concern as this suggests that the irrigation system may act as an inoculum source for future crops unless clean-up is comprehensive. It also implies that the use of the NatuGro program, which is fed through the irrigation system, was not able to prevent infection within the system. The NatuGro program may have played a disease suppression role, but further work would be required to demonstrate if this is the case.

Environmentally, 2018 was an unusual year for growers with light levels and temperatures well below usual in the spring. This was replaced with a prolonged period of hot and sustained sunny weather until mid-autumn. These conditions impacted how crops grew and may have influenced the root zone environment, potentially suppressing bacterial growth and / or symptom development. Although no two growing years are ever the same, it would be interesting to know how root mat disease would have developed in 2018 under more 'normal' environmental conditions.

Greater levels of root mat disease occurred in the plastic removal trial at Site 3 than Trials D and E. There were no differences in root mat disease incidence or severity between either of the two plastic removal treatments and the untreated control at any of the assessment date. Although this indicates that the removal of the plastic is ineffective, the overall level of infection was less than 4% and it is possible that differences could have developed if a greater level of infection had developed.

Despite only one visibly infected cube occurring in Trial A, infected roots were found to be present within the slab. Root mat disease will frequently occur in slabs, but this is accompanied with root mat presence on the cube. Site 1 removes the plastic from the surface of their slabs as standard and this may have had a suppressive role on this site. Further work, in an artificially inoculated trial may be better suited to establish if plastic removal has an effect.

Analysis of over 40 rhizogenic *R. radiobacter* strains showed differences in their genetic and phenotypic traits (Bosmans, L. 2015). This study demonstrated considerable variability in the environmental conditions which certain strains were shown to survive under, including temperatures (between 4 and 44°C) and pH (3-11). This larger than expected diversity may go some way in explaining why different results were found in the three 2017 trials and perhaps also the 2018 results. Trails B and C were both situated at Site 2 and may have been infected with the same isolate(s), having been sourced from the same Dutch propagator. If they were infected with the same strain(s) this would explain why both trials at Site 2 responded similarly to the use of Carbon Gold. In 2017 Site 1 sourced their plants from a different Dutch propagator to Site 2, and this isolate may have reacted differently to the components of Carbon Gold, resulting in no reduction in rot mat infection and the increases in incidence observed.

Certain isolates of *R. radiobacter* have been demonstrated to be catalase positive, and able to convert hydrogen peroxide to water and oxygen gas (Bosmans L., 2015, Bosmans L., 2016). Seven of 41 strains investigated were able to convert hydrogen peroxide, or to tolerate it to high levels. This is of specific concern to growers who use hydrogen peroxide based

biocides at crop turn around, such as Endosan and Geosil, which may result in enrichment of the *Rhizobium* populations for catalase positive types. Some of the isolates tested were able to survive at 300ppm and a few as high as 600ppm, which is above the shock concentrations recommended by the manufacturers.

Over three quarters of these strains formed biofilms. This trait combined with an ability to break down, or withstand hydrogen peroxide at high concentrations risks the persistence of rhizogenic *R. radiobacter* strains between crops. It is likely that the few strains of *R. radiobacter* able to withstand the high concentrations arose directly from a high selective pressure from the use of hydrogen peroxide. Continued use, or insufficient concentrations / duration of use may select for these isolates. This would make treatment with hydrogen peroxide based products, for root mat disease, ineffective. Several Dutch and UK based propagators were requested to provide information on their clean-down practices between crops. Almost all that replied confirmed that they used hydrogen peroxide biocides. As a result of this, strains arriving at sites may already be less sensitive to these biocides.

Tomato root mat infection may arise from two sources, from a pre-existing source on site or via introduction from a propagator. If hydrogen peroxide treatment is a standard disinfectant, any isolates introduced may already have some ability to convert or tolerate hydrogen peroxide to some extent. This will make elimination of these isolates even harder.

Additional observations: Potential influence of the irrigation system on root mat incidence and severity

During 2017 heat maps of an area of cv. Funtelle treated with a greater rate of Vitix at Site 2 revealed an unusual distribution of root mat disease incidence. At the mid-crop and end-of-cropping assessments a much larger incidence of root mat disease was found in the one half of the eight rows assessed compared to the other. This area was not part of the trial and as such no statistical analysis was performed and these observations were anecdotal in nature.

R. radiobacter is known to form biofilms which can protect both *R. radiobacter* and other microorganisms from biocides / disinfectants. Earlier work in this project has demonstrated that several commonly used biocides within the industry were insufficient to completely eradicate biofilms of *R. radiobacter* following 10 minutes of treatment. It is possible that clean down at turnaround was insufficient in this additionally treated area and *R. radiobacter* and pRi persisted between crops within the irrigation system. *R. radiobacter,* and other microorganisms, could be transported during the early irrigation events directly to young susceptible plants. Irrigation systems can accumulate substantial amounts of biofilms and organic matter over the course of a season. Areas at the ends of the irrigation system, including end-caps, are those which are most likely to contain ‘active’ biofilms as the

concentration of biocide active here, e.g. hydrogen peroxide or hypochlorous acid, will be reduced compared to that entering the system.

At planting, over a third of all plants included in Trial F had a pinch of their roots collected and sent away in four composite bags for qPCR analysis by Fera Science Ltd. All four composite samples tested negative for bacterial presence and root mat infection.

At the first symptom assessment three months later 12 cubes in two plots (plots 21 and 23) showed visible infection (Figure 13). Ten of these twelve infected cubes were located in one plot (plot 21). The rows immediately following plot 24 were also walked and visible root mat infection was observed in several. This was sporadic and no rows showed root mat disease incidence to the extent of plot 21. No visible infection was recorded in plots 1 – 20.

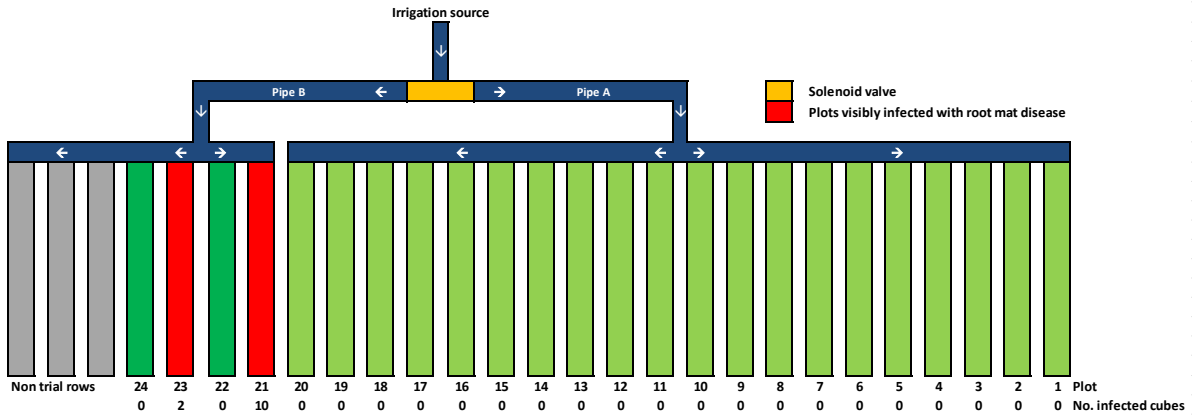

Figure 14. Layout of the irrigation system supplying Trial F, including the number of plots with visible symptoms of root mat disease and the number of infected cubes within those plots – Site 3, April 2018

The irrigation system providing the compartment housing Trial F splits after a solenoid valve with one pipe (Pipe A) supplying the majority of plots within the trial and the preceding rows to the south wall. Another pipe (Pipe B) supplies the remaining trial plots on the north side of the glasshouse (Figure 13). Pipe A supplies water to plots 1 - 20 where no visible infection was recorded at the first-symptom assessment. Pipe B supplies plots 21 - 24 which contained every incidence of the disease recorded at this date. This pipe also irrigates the plots beyond plot 24 which also contained visibly infected cubes. The end of plot 21 represents the furthest point from the source of the irrigation system. As a consequence of this this area may experience a reduced concentration of biocide active compared to other components of the system.

It is possible to be confident with a high degree of certainty that the plants delivered to Trial F were free from root mat disease infection. The fact that infection was later found in the trial suggests that the disease arose from a pre-existing inoculum source on site, likely from within

the irrigation system. As only plots supplied by Pipe B were visibly infected at this time this suggests that infection arose due to an inoculum source remaining within the system supplying Pipe B and not that supplying Pipe A. Visible infection was found in two plots and a biofilm may have persisted within the piping feeding these two plots in particular.

One, or a few, trays containing infected plants could also explain why sections of infection were found in plots 21 and 23 and not the other plots. However, as such a large amount of plants / plot were randomly sampled (35% of the total number of plants) tested negative for infection and this is highly unlikely.

The qPCR testing of sampled roots from 10 plants / plot revealed that 12 plots were actually infected at the first-crop-assessment (Figure 14). This could be a consequence of very low levels of inoculum present in both areas fed by Pipes A and B which were insufficient to cause visible symptoms at this time, unlike the larger amount presumed to have been present in piping in plots 21 to 24.

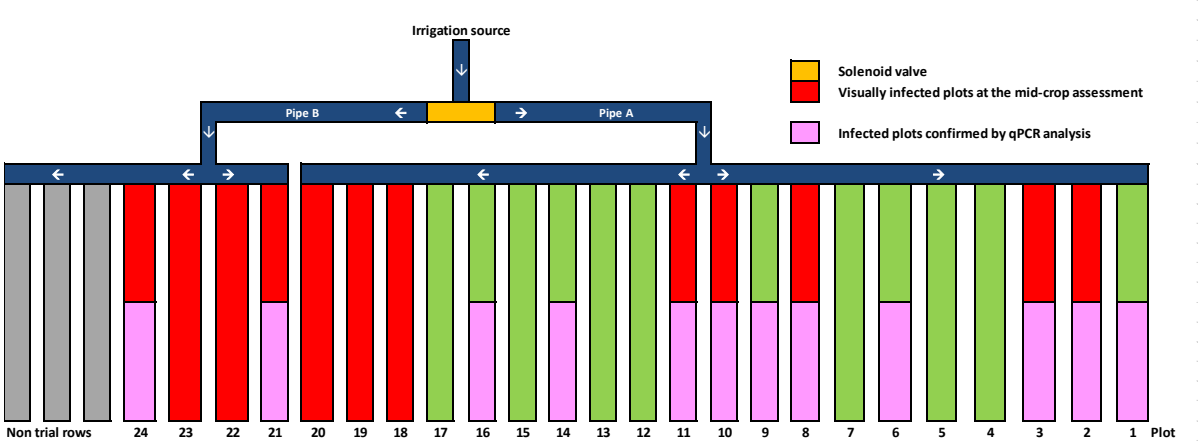

Figure 15. Layout of the irrigation system supplying Trial F, including the number of plots symptomatic of root mat disease which were shown to be infected via qPCR analysis (pink) and the plots which were visibly infected at the mid-crop assessment – Site 3, April 2018.

- Red and pink split plots are those confirmed to be infected via qPCR analysis that went onto develop visible infection by the mid-crop assessment.
- Green and pink split plots are those confirmed to be infected via qPCR analysis but did not go onto develop visible root mat symptoms by the mid-crop assessment.

This implies that site clean-up at crop turn-around was sufficient to reduce the level of *R. radiobacter* in the system to low enough levels to delay symptom development, but insufficient to complete kill it. Alternatively a new inoculum source was introduced following clean-up prior to the solenoid valve feeding Pipes A and B which infected plots across the whole trial area after planting. The high incidence of root mat disease in plots 21 and 23 were still likely due to pre-existing inoculum within the piping feeding them.

At the mid-crop assessment several plots were found to be visibly infected which had not tested positive by qPCR testing (Figure 14). Either these plants had become infected following sampling, or sampling roots from just 10 plants is insufficient to determine if a plot is infected. Testing more plants would be time-consuming and increase costs but may have revealed that many more, if not all, plots contained infected plants. Plots were also found which did test positive for infection at this time, but did not go onto develop visible symptoms by the mid-crop assessment.

At this stage it is impossible to ascertain the exact source of infection in Trial F. If infection did arise due to insufficient clean-up then this will need to be addressed and corrected. If this is the case it is entirely possible that root mat disease can be effectively eradicated from a tomato nursery if plants are delivered free of infection and all traces of *R. radiobacter* and pRi are removed from a site. This is much easier said than done and growers face unique challenges based on their individual infrastructure, equipment, local environmental conditions etc. and any reintroduction of infected material can rapidly undo these efforts. If *R. radiobacter* and pRi is being naturally reintroduced from outside the nursery from 'wild' sources then the local environment surrounding the business should be considered. It is unlikely that any measures to eradicate the bacteria from the natural environment will be possible however and focus should be on preventing entry of the bacteria into the nursery on plants / equipment etc.

In all cases the most effective biocides should be used, with growers considering the use of chlorine-based products e.g. Clorious2 which was the most effective biocide tested during the 2017 work and is effective against *R. radiobacter* strains which are catalse positive and able to tolerate higher concentrations of hydrogen peroxide.

Conclusions

Sources of infection

- Seed and irrigation water sampled from one UK and two Dutch propagators providing plants for the five of the six commercial trials all tested negative for *R. radiobacter* and the pRi
- In both 2017 and 2018, several treated and / or untreated plants tested positive for pRi at plant arrival on commercial sites suggesting infection occurred at either propagation or during delivery
- In 2018 the delivery lorry, trolleys and site architecture were swabbed and tested negative for the presence of *R. radiobacter* and pRi at arrival at Site 2. Plant roots were demonstrated to be severely infected at this time suggesting infection did not occur during delivery

- Infection may have occurred in one trial from a pre-existing inoculum source in once-used bags. Do not re-use coir or rockwool slabs with a history of the disease, unless they have been disinfested (e.g. steaming) and shown to be free of *R. radiobacter* and pRi.
- Observations of a distinct distribution of root mat symptoms in a separate commercial area and in a 2018 trial suggest the irrigation system may play a role in disease occurrence

Infection

- Young tomato plants were successfully inoculated with *R. radiobacter* and both grafted and ungrafted plants appeared equally susceptible
- Natural wounds, such as the points of lateral root emergence, are likely sufficient for infection to occur
- A separate study over 40 rhizogenic strains has shown considerable variability in the environmental conditions which individual *R. radiobacter* strains are able to survive and grow under, including a broad temperature, pH range and levels of hydrogen peroxide
- Individual *R. radiobacter* isolates may respond differently to the biological products applied in this work explaining the inconsistencies in the results gained between sites in 2017

Detecting infected plants

- Fera Science Ltd. are in possession of a rapid molecular assay capable of confirming the presence of pRi T-DNA in plant roots
- This test is not dependent on the presence of *R. radiobacter* and can therefore bypass the enrichment step used in the conventional diagnostic, potentially making the test faster and less expensive
- Insufficient visible root mat disease developed in the two efficacy trials at Sites 1 and 2 during 2018 for statistical analysis to be performed, despite roots being severely infected in Trial E at arrival. Not all plants found to be infected by the Ri plasmid necessarily developed symptoms of root proliferation

Disease management treatments

- A number of non-conventional products applied to the root zone were observed to reduce the expression of root mat symptoms however no treatment was successful in eliminating root mat disease in tomato

- 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
- Carbon Gold reduced the incidence and severity of tomato root mat disease in two trials during 2017, by up to 50% incidence in one trial
- The most effective treatments tested were Carbon Gold applied at propagation alone and the same product applied both at propagation and at planting
- Vitix did not reduce the incidence of the disease but reduced the severity of the disease at one assessment
- In trial A the use of Vitix was associated with an increased incidence of root mat disease (nearly three-fold), compared to the untreated control, when applied in propagation alone, suggesting treatment at propagation is an important component of disease management
- Chlorine-based biocides were significantly more effective than hydrogen peroxide-based biocides at killing catalase-positive *R. radiobacter* at the concentrations and exposure times tested. Chlorius2 can be used to treat irrigation water during crop production
- The chlorine dioxide-based biocide (Clorious2) effectively killed *R. radiobacter* as cells suspended in water at concentrations that are known not to be phytotoxic or corrosive, but further evaluation is needed to assess the dose / exposure time required to effectively remove viable bacteria in biofilms that can form within irrigation systems
- Removal of the plastic sleeve surrounding the cube and / or plastic from the surface of the slab was found to have no effect on root mat incidence or severity. Incidence remained low and differences may have been seen if a greater incidence of root mat had occurred
- There continue to be a number of knowledge gaps regarding *R. radiobacter*, the Ri plasmid and the transfer of T-DNA

End of season clean-up

- A newly formulated sodium hypochlorite product (Domestos Extended Power) was particularly effective and was the only product that eliminated viable *R. radiobacter* in biofilms on irrigation tubing within the experimental conditions used
- Further evaluation will be needed to determine the minimal effective concentration of the Domestos formulation and assess potential phytotoxic and corrosive effects

Knowledge and Technology Transfer

2016 Presentation of results to the Tomato Study Group, Fenstanton, Cambs, October 2016 (S Mayne)

2017 Presentation of results to the Tomato Study Group, Isle of Wight, June 2017 (D Kaye)

Presentation at the Tomato Grower Association Annual Conference, Kenilworth, September 2017 (D Kaye)

2018 Presentation of results at the Tomato Working Party, Hull, January 2018 (D Kaye)

Presentation of results to the Tomato Study Group, Isle of Wight, September 2018 (D Kaye)

Presentation of final results to the Tomato Working Party, Teesside, November 2018 (D Kaye)

2019 Presentation at Protected edibles Vine Cropping day, Rugby, February 2019 (D Kaye)

Articles submitted to the AHB Grower magazine (2016 - 2018)

Glossary

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

Opines – low molecular weight novel metabolites synthesised in plant tissues following the 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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Van Kerckhove S., (2015). Research insights into crazy roots (root mat disorder) in tomato. Grodan Green Expert Platform meeting, Delft, The Netherlands, 11 February 2015.

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Factsheets

PE 029: Protected tomato – A review of root mat and crown gall diseases to inform research on control of tomato root mat -

https://horticulture.ahdb.org.uk/sites/default/files/research_papers/PE%20029_Review%20document%20%2810June16%29.pdf

CP 174: Review of bacterial pathogens of economic importance to UK crops - https://horticulture.ahdb.org.uk/sites/default/files/research_papers/CP%20174_Report_Final_2017_0.pdf

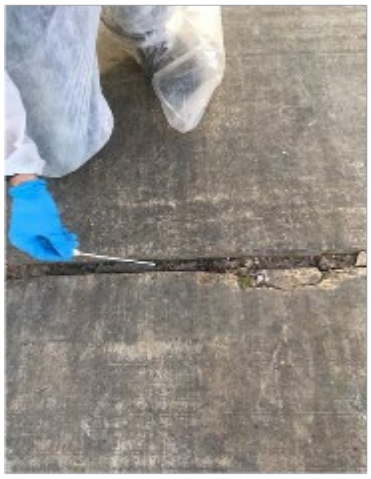
Appendices

Appendix 1 – Locations swabbed for qPCR determination of *R. radiobacter* and pRi presence at plant arrival on Site 2 – February 2nd 2018

1-8

9 & 10

11



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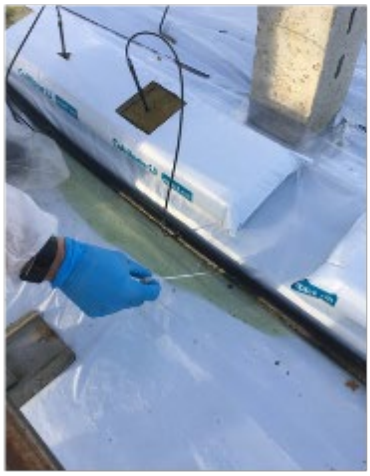

15

1. Lorry (left wall)
2. Lorry (left wall)
3. Lorry (left back corner, wall)
4. Lorry (floor left hand side and back)
5. Lorry (back wall)
6. Lorry (floor by wall)
7. Lorry floor (front)
8. Lorry tail lift floor
9. Piccolo trolley 1
10. Piccolo trolley 2
11. Leaking irrigation valve (valve 29)
12. Concrete pathway gap between plots 6 and 15
13. Trolley 27
14. Exposed soil at edge of glasshouse
15. Pooled (greening) water on new polythene

Appendix 2 – Root mat heat maps for final assessments of Trials D, E and F - 2018

Trial D – October 2018

Treatment List – Trial D

Trt	Product	a.i.	Rate	Timing
1	Untreated	-	-	-
2	Untreated	-	-	-
3	Carbon Gold	Biochar + biology blend	5g in propagation	at propagation
4*	Carbon Gold	Biochar + biology blend	55g at planting	At planting
5*	Carbon Gold	Biochar + biology blend	5g at propagation, 55g at planting	In propagation & at planting
6	Carbon Gold and Vitix (reduced rate)	Mixed inoculation	Vitix - 0.15g per m^2 at propagation in Carbon Gold propagation - 5g at only propagation	

Trt	Score
0	No root mat symptoms
1	1 or 2 upright roots on the cube 3-10 upright roots; at least 5% of cube surface
2	6-10% cube surface affected
3	10-20% cube surface affected
4	21-50% of cube surface affected
5	Over 50% affected; cube swelling
6	

Trial E – October 2018

Treatment List – Trial E

Treatment	Product	a.i.	Rate	Timing
1	Untreated	-	-	-
2	Untreated	-	-	-
3	Carbon Gold	Biochar + biology blend	55g between the cube and the slab	At planting

Trt	Score
0	No root mat symptoms
1	1 or 2 upright roots on the cube
2	3-10 upright roots; at least 5% of cube surface
3	6-10% cube surface affected
4	10-20% cube surface affected
5	21-50% of cube surface affected
6	Over 50% affected; cube swelling

LHS of central pathway (facing away from the door)

Plot	Cube																																																																															Trt
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

RHS of central pathway (facing away from the door)

Plot	Cube																																																																															Trt
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	2
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	3
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2

Treatment List – Trial F

Trt.	
1	Untreated
2	Untreated
3	Propagation cube sleeve plastic removal
4	Propagation cube sleeve and slab surface plastic removal

Trt	Score
0	No root mat symptoms
1	1 or 2 upright roots on the cube
	3-10 upright roots; at least 5% of
2	cube surface
3	6-10% cube surface affected
4	10-20% cube surface affected
5	21-50% of cube surface affected
6	Over 50% affected; cube swelling

Appendix 3 – qPCR results from root sampling at the first-symptom and end-of-cropping assessments for the three 2018 Trials D, E and F.

Trial D, Fist-symptom assessment - July 2018

	Asymptomatic Roots			Visibly Symptoms Roots		
Plot	Pre-Enrichment	Post-Enrichment	Direct Extraction	Pre-Enrichment	Post-Enrichment	Direct Extraction
1	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
2	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
3	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
4	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
5	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
6	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
7	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
8	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
9	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
10	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
11	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
12	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
13	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
14	40.00	40.00	**30.60**	-	-	-
	40.00	40.00	**32.07**	-	-	-
15	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
16	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
17	40.00	40.00	40.00	40.00	40.00	40.00
	40.00	40.00	40.00	40.00	40.00	40.00
18	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
19	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
20	40.00	40.00	40.00	-	-	-

	40.00	40.00	40.00	-	-	-
21	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
22	40.00	40.00	40.00	40.00	40.00	40.00
	40.00	40.00	40.00	40.00	40.00	40.00
23	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
24	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
25	40.00	**36.09**	**36.63**	-	-	-
	40.00	**37.03**	**36.34**	-	-	-
26	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
27	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
28	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
29	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
30	40.00	**34.84**	**32.51**	-	-	-
	40.00	**35.03**	**32.48**	-	-	-
31	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
32	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
33	40.00	**32.38**	**36.46**	-	-	-
	40.00	**33.00**	40.00	-	-	-
34	40.00	40.00	40.00	40.00	40.00	40.00
	40.00	40.00	40.00	40.00	40.00	40.00
35	40.00	40.00	40.00	**30.73**	**28.21**	**20.48**
	40.00	40.00	40.00	**31.04**	**28.06**	**20.46**
36	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-

- No visibly symptomatic cubes were present and no sampling was performed

Values in bold tested positive for *R. radiobacter* and / or root transformation via the Ri plasmid

Trial D, End-of-cropping assessment - November 2018

	Asymptomatic Roots			Visibly Symptomatic roots		
Plot	Pre-Enrichment	Post-Enrichment	Direct Extraction	Pre-Enrichment	Post-Enrichment	Direct Extraction
1	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
2	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
3	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
4	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
5	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
6	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
7	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
8	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
9	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
10	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
11	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
12	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
13	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
14	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
15	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
16	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
17	40.00	**32.30**	40.00	-	-	-
	40.00	**31.28**	40.00	-	-	-
18	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
19	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
20	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
21	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-

22	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
23	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
24	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
25	40.00	40.00	**34.99**	-	-	-
	40.00	40.00	**34.90**	-	-	-
26	40.00	40.00	**34.85**	-	-	-
	40.00	40.00	40.00	-	-	-
27	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
28	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
29	40.00	**30.74**	**32.85**	-	-	-
	40.00	**30.87**	**34.89**	-	-	-
30	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
31	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
32	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
33	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
34	40.00	40.00	40.00	-	-	-
	40.00	40.00	**36.18**	-	-	-
35	40.00	**32.24**	**33.76**	**32.84**	**21.38**	**20.27**
	40.00	**33.50**	**32.93**	**32.36**	**21.90**	**20.48**
36	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-

- No visibly symptomatic cubes were present and no sampling was performed

Values in bold tested positive for *R. radiobacter* and / or root transformation via the Ri plasmid

Trial E, Fist-symptom assessment - June 2018

	No symptoms observed			Rootmat Symptoms observed		
Plot	Pre-Enrichment	Post-Enrichment	Direct Extraction	Pre-Enrichment	Post-Enrichment	Direct Extraction
1	40.00	**38.89**	**35.00**	-	-	-
	40.00	40.00	**36.98**	-	-	-
2	40.00	**32.63**	**26.83**	**29.52**	**24.24**	**20.69**
	40.00	**32.37**	**26.92**	**29.81**	**23.02**	**20.78**
3	40.00	40.00	**33.30**	-	-	-
	40.00	40.00	**32.96**	-	-	-
4	**34.16**	**24.89**	**22.36**	-	-	-
	34.00	**24.57**	**22.25**	-	-	-
5	**32.00**	**22.82**	**20.88**	-	-	-
	32.56	**22.37**	**20.78**	-	-	-
6	40.00	**29.75**	**25.83**	-	-	-
	40.00	**29.87**	**26.07**	-	-	-
7	40.00	40.00	**29.35**	-	-	-
	40.00	40.00	**29.46**	-	-	-
8	40.00	**36.59**	**29.94**	-	-	-
	40.00	**36.96**	**30.15**	-	-	-
9	40.00	40.00	40.00	**35.49**	**26.67**	**25.32**
	40.00	**38.83**	40.00	**34.27**	**26.62**	**25.54**
10	40.00	**38.77**	**29.76**	-	-	-
	40.00	**37.75**	**29.72**	-	-	-
11	40.00	**38.69**	**26.16**	**31.53**	**30.44**	**23.18**
	40.00	40.00	**25.90**	**31.71**	**30.37**	**23.34**
12	**37.03**	**26.01**	**22.82**	-	-	-
	38.14	**25.92**	**22.79**	-	-	-
13	**38.58**	**36.94**	**28.52**	-	-	-
	38.66	**36.81**	**28.64**	-	-	-
14	40.00	**33.69**	**28.73**	-	-	-
	40.00	**32.97**	**28.63**	-	-	-
15	40.00	**34.35**	**28.25**	-	-	-
	40.00	**33.99**	**28.27**	-	-	-
16	**36.67**	**32.08**	**23.01**	-	-	-
	37.64	**31.14**	**22.90**	-	-	-
17	**35.58**	**26.99**	**24.64**	-	-	-
	35.97	**24.45**	**24.50**	-	-	-
18	**38.72**	**35.05**	**26.84**	-	-	-
	40.00	**35.55**	**26.69**	-	-	-

- No visibly symptomatic cubes were present and no sampling was performed

Values in bold tested positive for *R. radiobacter* and / or root transformation via the Ri plasmid

Trial E, End-of-cropping assessment - November 2018

	Asymptomatic Roots			Visibly Symptomatic roots		
Plot	Pre-Enrichment	Post-Enrichment	Direct Extraction	Pre-Enrichment	Post-Enrichment	Direct Extraction
1	40.00	40.00	**30.99**	-	-	-
	40.00	40.00	**30.88**	-	-	-
2	**30.40**	**18.27**	**28.35**	-	-	-
	30.75	**18.27**	**28.26**	-	-	-
3	40.00	**27.37**	**25.92**	-	-	-
	36.25	**27.08**	**26.00**	-	-	-
4	**34.31**	**31.92**	**28.45**	-	-	-
	37.52	**31.89**	**28.47**	-	-	-
5	40.00	40.00	**34.63**	-	-	-
	40.00	40.00	**35.58**	-	-	-
6	**33.00**	**33.04**	**24.97**	-	-	-
	33.18	**32.19**	**24.84**	-	-	-
7	**34.87**	**32.30**	**26.70**	-	-	-
	31.82	**33.11**	**26.75**	-	-	-
8	40.00	**31.79**	**32.16**	**36.36**	**19.44**	**21.71**
	40.00	**31.69**	**32.06**	**34.31**	**19.22**	**20.90**
9	**33.56**	**34.61**	**28.76**	-	-	-
	34.72	**32.01**	**28.95**	-	-	-
10	40.00	**37.13**	**29.38**	-	-	-
	40.00	**35.76**	**29.35**	-	-	-
11	**34.47**	**35.05**	**29.30**	-	-	-
	34.00	**34.80**	**29.39**	-	-	-
12	40.00	**32.35**	**31.59**	**36.45**	**24.58**	**23.33**
	40.00	**31.96**	**31.84**	**37.02**	**24.81**	**22.90**
13	40.00	40.00	**34.06**	-	-	-
	40.00	40.00	**33.75**	-	-	-
14	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
15	40.00	40.00	**32.82**	-	-	-
	40.00	40.00	**32.64**	-	-	-
16	40.00	**32.18**	**36.56**	**28.49**	**19.92**	**22.12**
	40.00	**31.50**	**35.95**	**29.24**	**19.96**	**22.20**
17	**35.21**	**31.53**	**30.96**	-	-	-
	35.83	**32.65**	**31.00**	-	-	-
18	40.00	40.00	**30.79**	-	-	-
	40.00	40.00	**30.66**	-	-	-

- No visibly symptomatic cubes were present and no sampling was performed

Values in bold tested positive for *R. radiobacter* and / or root transformation via the Ri plasmid

Trial F, Fist-symptom assessment - April 2018

	No symptoms observed			Rootmat Symptoms observed		
Plot	Pre-Enrichment	Post-Enrichment	Direct Extraction	Pre-Enrichment	Post-Enrichment	Direct Extraction
1	40.00	40.00	40.00	-	-	-
	40.00	40.00	**39.87**	-	-	-
2	40.00	40.00	40.00	-	-	-
	40.00	40.00	**35.92**	-	-	-
3	40.00	40.00	40.00	-	-	-
	40.00	40.00	**36.32**	-	-	-
4	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
5	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
6	40.00	40.00	40.00	-	-	-
	40.00	40.00	**35.86**	-	-	-
7	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
8	40.00	40.00	40.00	-	-	-
	40.00	40.00	**35.85**	-	-	-
9	40.00	40.00	40.00	40.00	40.00	40.00
	40.00	40.00	**35.41**	40.00	40.00	40.00
10	40.00	40.00	**37.04**	-	-	-
	40.00	40.00	40.00	-	-	-
11	40.00	40.00	**36.78**	-	-	-
	40.00	40.00	**35.19**	-	-	-
12	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
13	40.00	**36.12**	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
14	40.00	40.00	40.00	-	-	-
	40.00	40.00	**36.29**	-	-	-
15	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
16	40.00	40.00	40.00	-	-	-
	40.00	40.00	**35.92**	-	-	-
17	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
18	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
19	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
20	40.00	40.00	40.00	40.00	40.00	**35.94**
	40.00	40.00	40.00	**37.00**	**36.74**	40.00
21	40.00	40.00	**31.55**	**26.58**	**21.69**	**18.34**
	40.00	40.00	**31.74**	**26.72**	**21.04**	**18.21**
22	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
23	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
24	40.00	40.00	**36.78**	**27.79**	**20.42**	**18.80**
	40.00	40.00	40.00	**27.82**	**21.15**	**18.83**

- No visibly symptomatic cubes were present and no sampling was performed

Values in bold tested positive for *R. radiobacter* and / or root transformation via the Ri plasmid

Trial F, end-of-cropping assessment - November 2018

	Asymptomatic Roots			Visibly Symptomatic roots		
Plot	Pre-Enrichment	Post-Enrichment	Direct Extraction	Pre-Enrichment	Post-Enrichment	Direct Extraction
1	**36.22**	40.00	**31.92**	-	-	-
	40.00	40.00	**31.87**	-	-	-
2	**35.09**	**32.52**	**35.87**	**30.18**	**17.89**	**19.85**
	34.80	**33.08**	**34.80**	**28.63**	**18.02**	**20.10**
3	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
4	40.00	**34.67**	40.00	-	-	-
	40.00	**33.45**	**35.80**	-	-	-
5	40.00	40.00	**33.72**	-	-	-
	40.00	40.00	**33.74**	-	-	-
6	40.00	**35.04**	**35.41**	-	-	-
	40.00	**34.03**	**35.60**	-	-	-
7	40.00	40.00	40.00	**28.00**	**24.98**	**17.70**
	40.00	**36.60**	40.00	**28.47**	**25.35**	**17.90**
8	40.00	**34.76**	**26.79**	**28.94**	**19.85**	**18.63**
	40.00	**36.36**	**27.10**	**29.20**	**19.75**	**18.49**
9	40.00	**29.62**	**26.95**	**28.66**	**20.70**	**17.40**
	40.00	**29.33**	**27.33**	**28.51**	**21.74**	**17.64**
10	**36.30**	**34.86**	**32.15**	**26.94**	**18.74**	**17.37**
	33.91	**33.89**	**32.17**	**26.94**	**17.95**	**17.54**
11	40.00	40.00	**37.16**	**29.60**	**19.59**	**23.21**
	40.00	40.00	**35.62**	**29.57**	**19.56**	**23.14**
12	40.00	40.00	40.00	-	-	-
	40.00	40.00	40.00	-	-	-
13	40.00	40.00	**34.91**	-	-	-
	40.00	40.00	**34.31**	-	-	-
14	**33.86**	**34.22**	**25.04**	-	-	-
	34.26	**34.94**	**25.48**	-	-	-
15	**35.53**	**29.14**	**27.38**	-	-	-
	34.42	**29.26**	**27.96**	-	-	-
16	40.00	**36.51**	40.00	-	-	-
	40.00	**35.28**	**35.52**	-	-	-
17	**35.51**	**33.26**	**29.79**	**30.07**	**21.10**	**18.64**
	38.13	**32.34**	**29.46**	**29.77**	**20.76**	**18.81**
18	**33.48**	**20.28**	**23.39**	**31.69**	**16.85**	**18.61**
	33.48	**20.22**	**23.52**	**30.76**	**16.99**	**18.57**
19	**28.37**	**20.47**	**20.18**	**28.47**	**21.69**	**19.52**
	28.24	**21.62**	**20.12**	**24.74**	**21.81**	**19.50**
20	40.00	**29.74**	**31.44**	**28.37**	**19.86**	**19.33**
	40.00	**29.76**	**31.61**	**28.45**	**19.98**	**19.42**
21	**28.68**	**20.53**	**21.20**	**28.73**	**21.49**	**18.71**
	28.97	**20.56**	**21.24**	**28.78**	**21.52**	**18.69**
22	**29.80**	**20.87**	**22.66**	**27.60**	**18.30**	**17.80**
	29.87	**20.77**	**22.45**	**27.29**	**18.06**	**17.60**
23	**32.97**	**23.16**	**26.59**	**30.74**	**18.96**	**18.49**
	32.80	**23.34**	**26.69**	**30.58**	**19.28**	**18.72**
24	40.00	**26.85**	**30.62**	**27.57**	**19.56**	**19.02**
	36.06	**26.55**	**30.20**	**26.82**	**19.84**	**18.88**

- No visibly symptomatic cubes were present and no sampling was performed

Values in bold tested positive for *R. radiobacter* and / or root transformation via the Ri plasmid

Appendix 4 – Temperature and humidity graphs for Trials D, E and F from planting to the trials conclusion, January-November (Trial A), January to November (Trial E), January to November (Trial F).