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:	Annual report, December 2017
Previous report:	Annual report, December 2016
Key staff:	Tim O'Neill (project consultant) Aoife O'Driscoll & Vasiliki Tzortzi - ADAS John Elphinstone & Adam Bryning – Fera Science Ltd.
Location of project:	Commercial sites and Fera Science Ltd.
Industry Representative:	Dr Phil Morley, British Tomato Growers' Association (Technical Officer) APS Group (Director of Agronomy)
Date project commenced:	1 January 2016
Expected completion date:	30 December 2018

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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.

Dave Kaye

Plant Pathologist

ADAS UK Ltd

Signature  Date 30-01-2018

John Elphinstone

Head Bacteriologist

Fera Science Ltd

Signature  Date 30-01-2018

Report authorised by:

Barry Mulholland

Horticulture Team Manager

ADAS UK Ltd

Signature  Date 30-01-2018

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GROWER SUMMARY

Headline

- Carbon Gold applied at propagation alone and at propagation and planting reduced the incidence and severity of root mat disease in two of three trials
- Carbon Gold treated at propagation alone reduced the incidence of root mat disease by 50% in one trial.
- Vitix reduced the severity of root mat at only one assessment, and significantly increased the incidence and severity of root mat disease compared to the untreated control in one trial
- 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 *Rhizobium radiobacter* at the concentrations and exposure times tested
- A newly formulated sodium hypochlorite product (Domestos Extended Power) was the only product that eliminated viable *R. radiobacter* in biofilms on irrigation tubing within the experimental conditions used
- The chlorine dioxide-based biocide (Clorious$_2$) effectively killed *R. radiobacter* as cells suspended in water at concentrations that are known not to be phytotoxic or corrosive

Diagnostics:

- A molecular diagnostic validated during 2016 was used in 2017 to identify tomato roots transformed by incorporation of the *Rhizobium cucumopine* Ri plasmid (i.e. to confirm infection by the root mat disease bacterium)
- Symptomless infected plants were detected at plant arrival on commercial sites suggesting infection occurred prior to arrival on site
- Symptomless infection was also detected within crops; the first visually symptomatic plants were observed around 13 - 16 weeks following planting

Background

Root mat disease of tomato caused by strains of *Rhizobium radiobacter* carrying a root-

inducing (Ri) plasmid is an increasing problem in the UK and around the world. Current knowledge of disease biology and crop observations both indicate that infection probably occurs when plants are young, including during propagation, though symptoms can take many weeks to develop. Work in this project has confirmed that young tomato plants do arrive on commercial sites infected with *R. radiobacter*. The disease causes excessive vegetative growth, reduced fruit size and quality, whilst increasing the risk of secondary root rot development. Together these result in significant crop losses estimated at 15% including additional management costs.

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

This project concentrates on management of root mat disease by both prevention of infection and reduction in 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 review produced for Objective 1 can be accessed as a separate document available from the AHDB website and is due to be updated in 2018.

During 2017, biological-based products selected from work carried out in 2016 were applied at propagation alone, in crop alone, or a combination of application in propagation and in crop, in three commercial tomato trials, to determine their effect in reducing the incidence and severity of root mat disease (Objectives 3 and 4). The efficacy of several commonly used biocides used at crop turnaround were also assessed for their ability to reduce *R. radiobacter* and Ri plasmid populations in a lab-based study at Fera Science Ltd.

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 by bacteria carrying

the root initiation plasmid (pRi) and 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. This review will be updated during 2018 to include recent research and grower inputs.

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 developed in 2016 by Fera Science Ltd. Complete information for 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

Out of eight treatments tested during 2016 Carbon Gold, Trianum P and Serenade ASO were found to reduce root mat disease. Full results can be found in the PE 029 2016 annual report. Further work on this objective carried out in 2017 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

Three commercial trials, Trials A, B and C, were carried out in 2017 to further examine post planting treatments (Objective 4) and product efficacy (Objective 3). Carbon Gold was chosen based on its performance during the 2016 trial work. A mixture of Trianum P and Serenade ASO could not be used as Serenade ASO is not approved as a drench treatment for use on protected tomato. Therefore, an alternative product, Vitix, containing both *Trichoderma* spp. and *Bacillus* spp. was chosen for the three commercial trials.

Plants were treated at propagation, at both propagation and in crop, and at crop alone as outlined in Table 1. It is believed that infection occurs either at the propagation stage or shortly after plant arrival on site and early treatment is vital for effective disease control. Carbon Gold is an insoluble biochar and as such only applied once at propagation and once at planting. Vitix is water soluble and was applied by hand drench to the plants requiring repeat application every eight weeks. Due to the fully randomised nature of the trial design, Vitix could not be applied via the drip lines as would be the case commercially.

Table 1. A summary of the timing and doses of the treatments used in the three commercial trials during 2017

Trt	Product	Treatment timing	Dose
1	Untreated	-	-
2	Untreated	-	-

3	Carbon Gold	Propagation	5 g, dusted around plug
4	Carbon Gold	Propagation	5 g, dusted around plug
		& at planting	55g between cube and slab
5	Vitix	Propagation	1.5 g/m^2
6	Vitix	In-crop	3 g/100 plants
7	Vitix	Propagation & in-crop	1.5 g/m^2 & 3 g/100 plants

Seed and water samples sent from the final irrigation timing at two Dutch propagators providing plants tested negative for root mat disease using the rapid qPCR assay developed by Fera Science Ltd. in 2016. Root samples from the three propagation treatments were tested upon arrival at each of the two nurseries (Sites 1 and 2). These revealed that treated plant material was infected with root mat disease (Table 2). Interestingly Trials B and C were sourced from the same Dutch propagator (though different varieties), with Trial B showing no signs of infection and Trial C showing infection in the treated plants alone. Treatments within each variety were kept separate during the propagation stage, if *R. radiobacter* and the Ri plasmid were present at the propagator in certain areas this may explain these unexpected results.

Table 2. A summary of the treatments which tested positive / negative for pRi T-DNA at plant arrival for each of the three trials

			Treatment		
Site	Trial	Propagator	Untreated	Carbon Gold	Vitix
1	Trial A	1	✓	×	×
2	Trial B	2	×	×	×
2	Trial C	2	×	✓	✓

✓ - Positive detection of pRi T-DNA, × - No detection of pRi T-DNA

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 (May-June), mid-cropping (July–September) and at end-of-cropping (November) for both the incidence and severity of the 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. The incidence and severity results for all treatments at the three assessment dates are located in Table 3.

At the first symptom assessment, no differences were found between any of the treatments and the untreated control in root mat incidence or severity in Trials B and C. Despite a low incidence of disease, a statistically significant increase in root mat incidence was seen in plants treated with Vitix at propagation alone in Trial A (0.4% affected in the untreated and 4.1% in Vitix treated plants). This was accompanied by an increase in severity scores of cubes exhibiting greater than 10% of surface root mat coverage.

Interestingly the qPCR results at the first-symptom assessment revealed that despite low numbers of visibly symptomatic plants, most plots in all three trials were infected with root mat disease. Although root samples from all treatments in Trial B tested negative for the disease at plant arrival in March 2017, all plots were infected by June 2017, likely from viable *R. radiobacter* containing the Ri plasmid in the once-used slabs from the 2016 season. Disease incidence in Trial B was much greater than in Trials A and C which supports this theory. The disease was also found in the treatments which tested negative on arrival in Trials A and C which were grown on new, unused slabs. It is likely that they became infected from either the disease introduced at plant arrival or a pre-existing inoculum source such as a *R. radiobacter* biofilm in the irrigation system.

The mid-crop assessment in Trial B gave promising results. Carbon Gold treated at propagation and at planting reduced the incidence of root mat disease from 43% in the untreated to 33%. (Table 3). No other reductions in incidence were seen at this assessment date.

A positive treatment effect was also seen in reducing 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 both Carbon Gold applied at both propagation and planting and Vitix applied at both propagation and in crop giving the greatest reductions in disease severity, reducing the proportion of cubes above 10% coverage from 9.5% to 4.0% and 4.2% respectively.

Table 3. Summary of treatment effects on root mat incidence and severity in commercial trials - 2017

Trial & treatment	Timing		% plants visibly infected			% plants with>10% cube area affected		
	Propagator	Crop	Early	Mid	Late	Early	Mid	Late
Trial A (cv. Piccolo, new coir slabs)								
1. Untreated	×	×	0.4	3.8	4.9	0.3	2.5	3.7
2. Carbon Gold	✓	×	0.6	4.8	8.3	0.4	2.6	3.9
3. Carbon Gold	✓	✓	0.8	3.3	3.7	0.4	2.2	2.4
4. Vitix	✓	×	4.1	13.3	13.6	3.3	7.6	10.5
5. Vitix	×	✓	0.8	9.8	9.4	0.6	6.7	7.0
6. Vitix	✓	✓	0.8	6.3	6.6	0.4	3.7	5.5
Trial B (cv. Piccolo, old coir slabs)								
1. Untreated	×	×	16.9	43.4	54.1	0.2	9.5	8.4
2. Carbon Gold	✓	×	15.3	42.5	49.0	0.2	**6.0**	7.5
3. Carbon Gold	✓	✓	17.2	**33.5**	**44.4**	0.0	**4.2**	5.8
4. Vitix	✓	×	14.1	44.4	55.2	0.0	9.8	10.8
5. Vitix	×	✓	12.2	40.8	47.8	0.0	**6.0**	9.3
6. Vitix	✓	✓	13.5	43.6	49.4	0.3	**4.0**	6.2
Trial C (cv. Funtelle, new coir slabs)								
1. Untreated	×	×	1.9	15.7	34.8	0.6	10.4	30.6
2. Carbon Gold	✓	×	2.2	15.2	**16.8**	1.9	10.5	**12.8**
3. Carbon Gold	✓	✓	1.7	11.9	28.9	1.9	7.2	**24.1**
4. Vitix	✓	×	1.4	16.6	29.6	0.0	10.5	26.9
5. Vitix	×	✓	2.0	15.2	29.2	0.0	9.0	25.7
6. Vitix	✓	✓	1.4	16.2	33.8	2.6	7.9	31.0

*Treatments that significantly reduced root mat disease compared to the untreated are emboldened
*Treatments that significantly increased root mat disease compared to the untreated are underlined

The final assessment in Trial A mirrored that of the earlier assessments with a statistical increase in both the disease incidence and severity of root mat disease in plants treated with Vitix in propagation alone and in crop alone. At this final assessment the incidence of root mat disease in the untreated was 4.9% compared to Vitix applied at propagation alone, 13.6% and in-crop alone, 9.4%. At this date an increase was also seen in plants treated with Carbon Gold at propagation alone. The proportion of severity scores above 10% cube coverage was similarly larger increasing from 3.7% in the untreated to 10.5% in plots treated with Vitix in propagation alone and 7.0% in those treated with Vitix in crop alone. No Carbon Gold treatment increased the root mat severity scores at this time.

Carbon Gold applied at propagation and at planting combined reduced the incidence of root mat disease from 54% in the untreated to 44% in Trial B by the final assessment. This was a similar reduction to that seen at the mid-crop assessment. No other differences in incidence and severity scores were seen between any treatments and the untreated at this assessment.

At the final assessment in Trial C, Carbon Gold applied at propagation alone reduced the

incidence of root mat by half from 34.8% in the untreated plots to 16.8% (Figure 1). Both Carbon Gold applied at propagation alone and Carbon Gold applied at both propagation and at planting combined also reduced the severity of the disease by significant amounts, 12.8% and 24.1% respectively compared to the untreated at 30.6%.

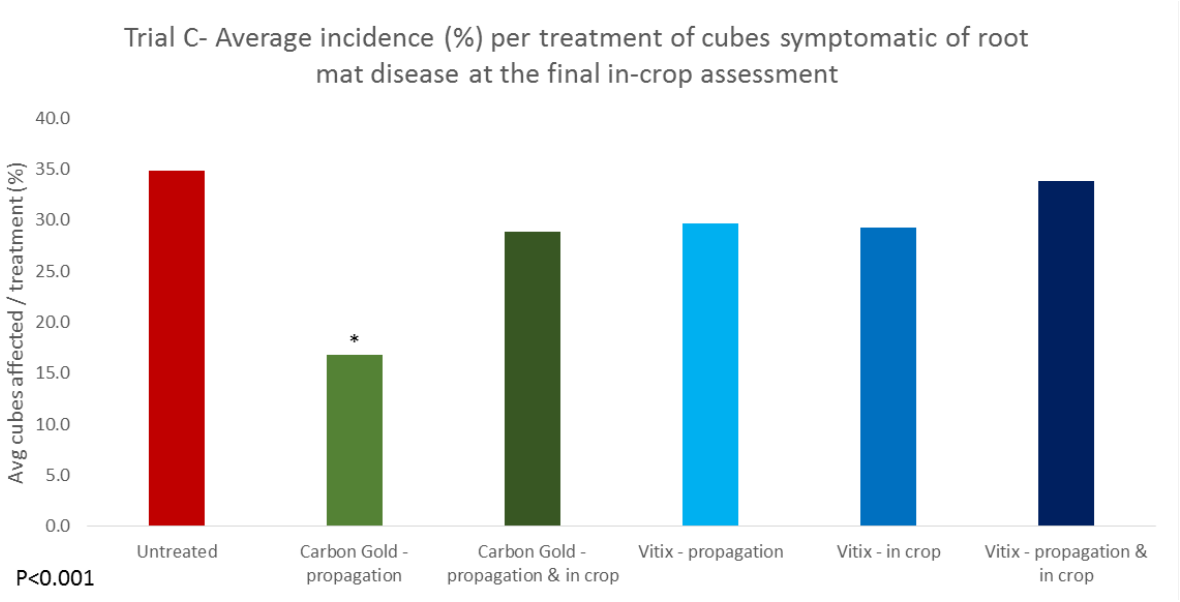


Figure 1 - Incidence (%) of root mat disease in treated and untreated plots in Trial C at the end-of-cropping assessment, 14th-16th November 2017
*Statistically different to the untreated control

Reductions in root mat disease incidence and severity were seen in Trials B and C situated at Site 2 with commercially significant reductions recorded at the mid-crop and end-of-cropping assessments. Both Carbon Gold treated at propagation alone and Carbon Gold treated at both propagation and at planting were shown to be effective in separate trials. Interestingly in Trial B at the mid-crop assessment, Carbon gold applied at propagation alone did not significantly reduce incidence when Carbon Gold applied at both timings did. These results suggest that application at propagation is an important treatment time, but it may be necessary to repeat the treatment at planting to see an effect. The opposite was seen at the final assessment in Trial C however, with Carbon Gold treated at both timings not having an effect on disease incidence where application at just propagation did. Future work looking at Carbon Gold in 2018, including a Carbon Gold in-crop treatment only will further assess this treatment and best time of application. Vitix, which performed poorly, increasing the disease incidence in one trial and only reducing the severity in one instance will not be carried forward.

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, Clorious$_2$, Geosil, Endosan 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 (Table 4). After mixing a subsample was removed following exposure to four different lengths of time (0, 2, 5 and 10 minutes) and grown onto nutrient dextrose agar at 28°C for 48 hours with growth of surviving bacteria recorded.

Table 4. Summary of the four biocides used in experiments 1 and 2 with manufacturers recommended doses for each product

Biocide / manufacturer	**Active ingredient (a.i.)**	**Recommended dose**	
		Water treatment	**Shock treatment**
Clorious$_2$ / Brenntag	Chlorine dioxide	0.75 ppm ClO_2	7.5ppm ClO_2
Geosil / GeoSIL Pacific Ltd.	Hydrogen peroxide and stabilized silver	50 ppm H_2O_2	500 ppm H_2O_2
Endosan3 (Endosan Enterprises (UK) Ltd.	Hydrogen peroxide and stabilized silver	50 ppm H_2O_2	500 ppm H_2O_2
Domestos Extended Power / Unilever UK	Sodium hypochlorite	1620 ppm NaOCl	1620 ppm NaOCl

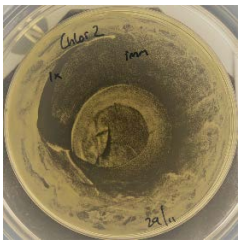
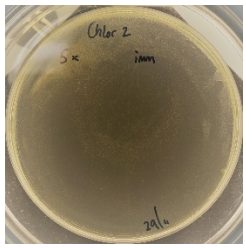
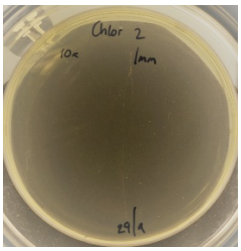
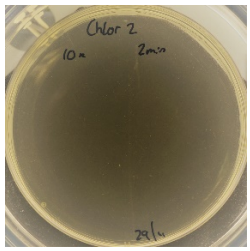
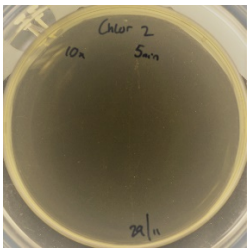
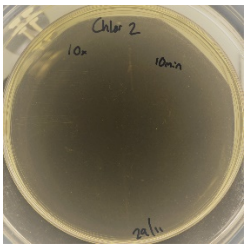
- *R. radiobacter* were challenged with biocide concentrations at 0.1x, 0.5x, 1x, 5x and 10x the recommended doses

At the dose rates studied, the most effective biocide was Domestos Extended Power, achieving 100% kill of the bacterium at all concentrations tested. Clorious$_2$ was 100% effective at half and full recommended doses, but only if the exposure time was two minutes or greater.

No effect of Clorious$_2$ was observed at 0.1x the recommended dose. Plate testing images for each of the biocide treatments at different rates / exposure times are located in Figure 2.

It is important to note that the recommended dose of Clorious$_2$ (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. Use of Domestos may also generate chlorates within the plant which Clorious$_2$ does not. 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. The health and safety aspect of use of Domestos at either of these stages would also have to be assessed.

The hydrogen peroxide (H_2O_2) / stabilised silver biocides Geosil and Endosan 3 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.

Clorious$_2$	0.075 ppm ClO_2 - 0 min 	0.375 ppm ClO_2 - 0 min 		
Clorious$_2$	0.75 ppm ClO_2 - 0 min 	0.75 ppm ClO_2 - 2 min 	0.75 ppm ClO_2 - 5 min 	0.75 ppm ClO_2 - 10 min 
Domestos	162 ppm NaClO - 0 min	810 ppm NaClO - 0 min	1620 ppm NaClO - 0 min	

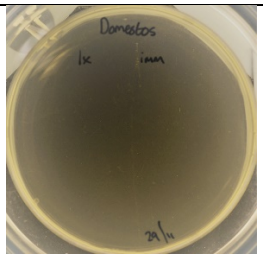
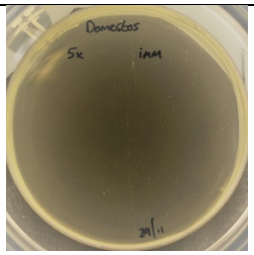
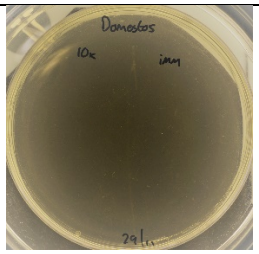
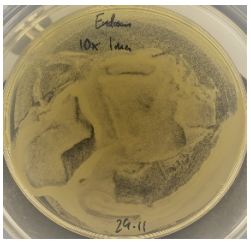
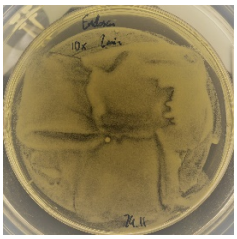
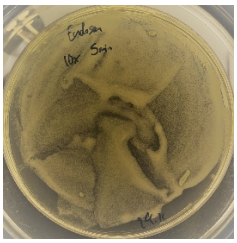
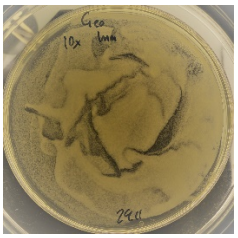
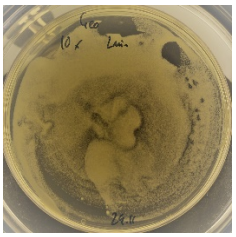
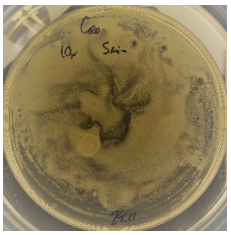
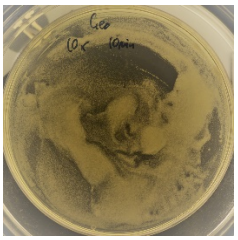
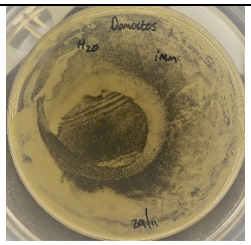
				
Endosan3	50 ppm H_2O_2- 0 min 	50 ppm H_2O_2- 2 min 	50 ppm H_2O_2- 5 min 	50 ppm H_2O_2- 10 min 
Geosil	50 ppm H_2O_2- 0 min 	50 ppm H_2O_2- 2 min 	50 ppm H_2O_2- 5 min 	50 ppm H_2O_2- 10 min 
H_2O				

Figure 2. Regrowth of *R. radiobacter* on nutrient dextrose agar following different biocide treatments

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

Segments of PVC dripper tubing (1 cm long) were coated in bacterial biofilm by dragging them through agar containing cultures of *R. radiobacter*. Each biocide was prepared at the shock treatment concentrations (Table 4), with the concentrations of ClO_2 and H_2O_2 verified using indicator strips before and after treatment. Contaminated tube segments were submerged in each biocide and exposed for 2, 5 or 10 minutes. Enrichment of any remaining viable bacteria was then performed for 72 hours at 26-28°C on a shaking incubator at 150 rpm. Any bacterial growth during enrichment was determined by performing qPCR TaqMan

analysis. Growth was indicated by a decrease in critical threshold (Ct) values when pre-and post-enrichment samples were compared. Lower Ct scores post-enrichment indicate an increase in populations of *R. radiobacter.*

Only Domestos Extended Power was effective at preventing further multiplication of *R. radiobacter* at the concentrations and exposure times tested (Figures 3-5). 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 activity of the biocides rather than that 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 two minutes exposure (as indicated by no significance between pre- and post-enrichment Ct values). Domestos may therefore be a potential candidate for end of season flushing of the irrigation line, though further work will be required to establish its practical suitability. Further investigation will also be needed to determine whether the other biocides can successfully remove viable biofilm by increasing doses and/or exposure times.

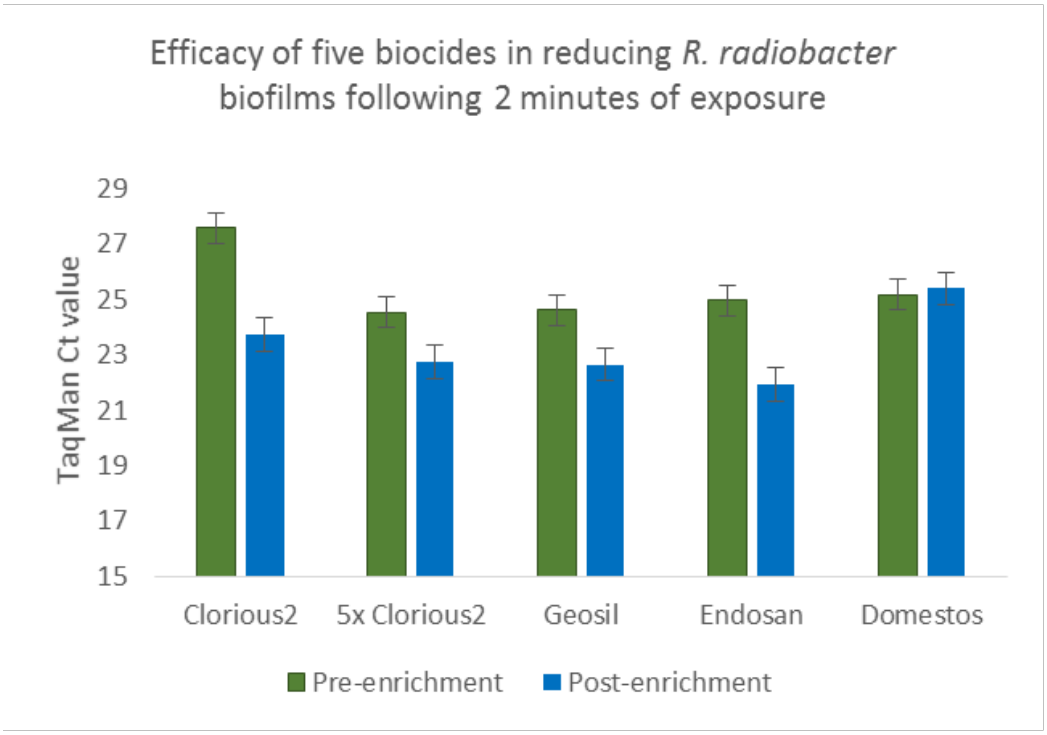


Figure 3. Ct scores for pre- and post-enrichment of *R. radiobacter* following a two minute exposure to four biocides – December 2017

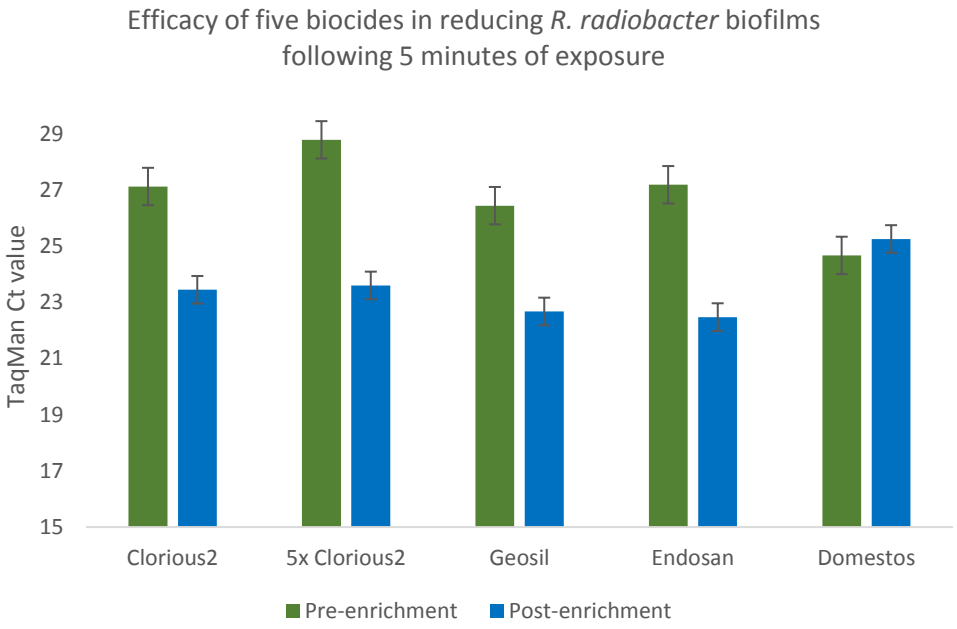


Figure 4. Ct scores for pre- and post-enrichment of *R. radiobacter* following a five minute exposure to four biocides – December 2017

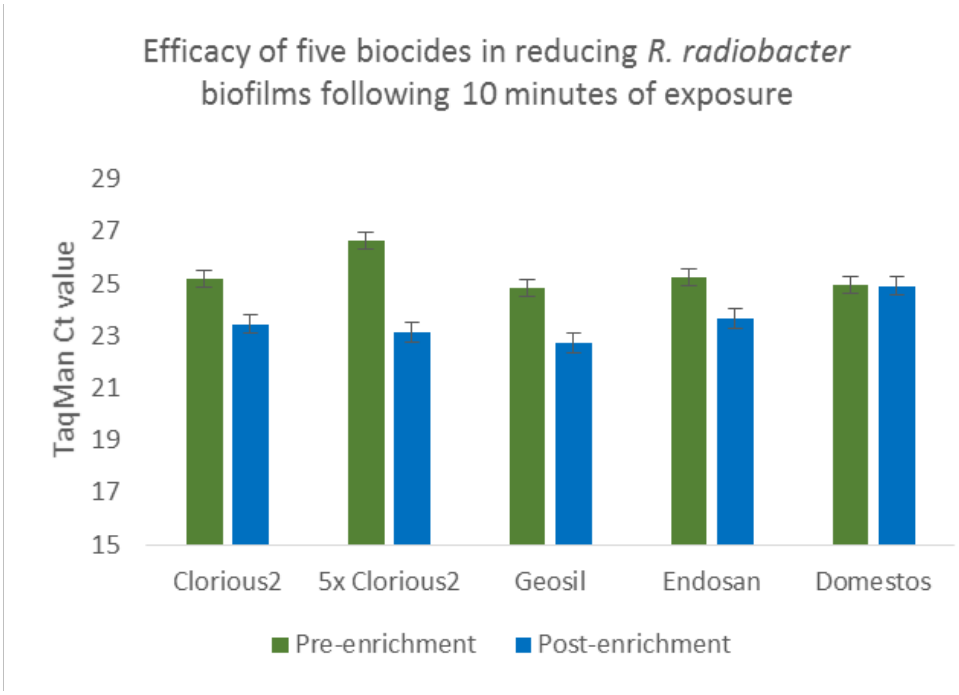


Figure 5. Ct scores for pre- and post-enrichment of *R. radiobacter* following a ten minute exposure to four biocides – December 2017

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

Work contributing to Objective 6 is ongoing throughout the duration of this three-year project. During 2017 results from the commercial trials were presented at a Tomato Study Group meeting in June and presented at the 2017 Tomato Gowers Association in September.

Additional observations

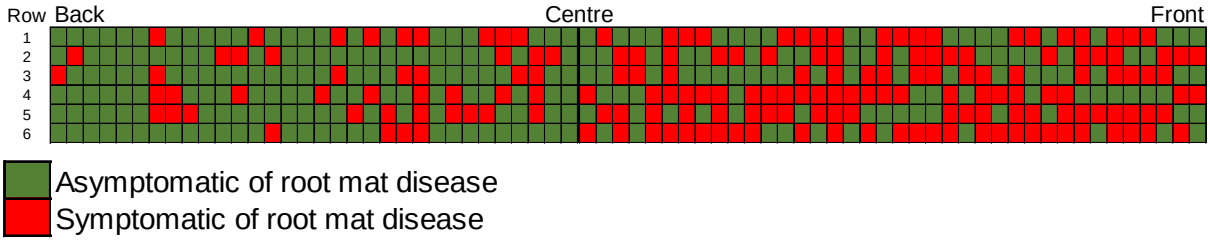
Potential impacts of the irrigation systems on spread and management of root mat disease - Six rows of cv. Funtelle at Site 2 was treated by the host grower with Vitix applied at a greater rate than in the 2017 trials (~1.5x) to see if there was an enhanced treatment effect. This area was assessed twice over the season in July and November. No statistical analysis was possible on this single treated block, but the observational outcomes may provide anecdotal evidence for future avenues of research.

Differences were seen in the distribution of affected plants along the length of the six rows assessed. The irrigation set-up in this compartment is such that the rows are irrigated from the front and back simultaneously (see irrigation plan in Appendix 4). All rows in the monitored area were fed by the same irrigation system. As a result of this, water destined for the back half of each plot has a greater distance to travel. At the start of the season irrigation frequency is low, with watering occurring as little as every few days. This results in water remaining in the irrigation system for long periods of time.

In the first half of each row, receiving water from the front, a much higher incidence of root mat disease was seen than in the second half of the rows. Incidence in the first half was 70 compared to 30 at the back in July and 123 compared to 54 in November (Figure 6). It has been proposed that dissolved oxygen levels in irrigation water may play a significant role in disease management. The water destined for the rear of the rows, which exhibited a lower level of root mat disease, had a greater distance to travel, but through leaking pipes and had a lower disease incidence. It is theorised that irrigation leaks may lead to greater oxygenation of the irrigation water which may be antagonistic to *R. radiobacter* biofilm development. The low irrigation frequency at plant arrival may result in low levels of dissolved oxygen within the system due to the formation of *R. radiobacter* biofilms. *R. radiobacter* bacterial cells may subsequently be transported in the system to infect young plants. One grower in Holland

continues to purge their entire system through clean-up to planting to maintain clean irrigation piping, reducing the risk of biofilm formation and maintaining good levels of water oxygenation. Future work comparing dissolved oxygen levels with root mat disease incidence could determine if this has an effect.

Figure 6. The distribution of root mat incidence across the six assessed rows of plants treated with a higher rate of Vitix at the end-of-cropping assessment, November 2017



Additional variety observations – Several varieties which differ in generative and vegetative growth were also grown on once-used coir slabs with a history of root mat disease, including Lyterno, Roterno, Elegance, Sweetelle and Vesuvius. These were observed at the mid-cropping and end of cropping assessments. All varieties suffered from root mat infection confirming all varieties were susceptible to root mat infection. Interestingly the generative varieties Lyterno and Roterno appeared to suffer most, with considerable root mat presence on the surface of the slabs and cubes. These severe infections led to significant issues with irrigation, with water running off the cubes / slabs, and not being absorbed into the coir. Marketable yield potential was reduced due to out of specification fruit and blossom end rot, any rotting fruit left on the vines also led to an increase in pest presence.

Additional coir from spare slabs was added on top of the matted roots to improve water uptake, often with additional irrigation pegs added and routine calcium sprays were performed to reduce the quantity of fruit suffering from blossom end rot. These plants arrived later than normal (in March). It is believed that more vegetative varieties typically suffer from root mat disease to a greater extent than generative varieties. Interestingly this contradicts what was seen during the monitoring at Site 2 with Lyterno and Roterno suffering the most severe symptoms. Although these measures did not eliminate the problem, they did allow the crop to be steered towards more normal growth with less fruit observed to suffer blossom end rot by the final assessment, likely increasing marketable yield. This demonstrates it can be possible to recover a crop severely infected with root mat disease to some extent using cultural methods.

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.
- As root mat does not commonly affect all plants in a crop evenly, crop steering becomes increasingly difficult as symptoms appear and the previously homogenous crop profile becomes randomly variable.

Action Points

- Any product applications designed to prevent infection, spread or development of symptoms of root mat should begin at the earliest stage possible e.g. at sowing or in propagation.
- As *R. radiobacter* is ubiquitous in the environment, good hygiene and sanitation practices should be followed throughout the year, especially at clean-up, and monitoring when and where symptoms occur each year may help identify areas where more effective clean-up is required.
- The testing of young plants using the rapid qPCR test developed by Fera Science Ltd. before transplanting may help prevent the introduction of infection.
- 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, once established, experience shows that root mat can spread quite readily from infected to healthy young plants.
- Carrying out a strict clean-up protocol at crop turnaround is considered ‘best practice’ and will help ensure *R. radiobacter* inoculum is 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 Clorious$_2$ 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 Endosan.
- 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 and is only recommended for the disinfection of surfaces at times such as end of season clean-up.

SCIENCE SECTION

Introduction

Root mat disease of tomato affects nearly 90% of UK tomato nurseries. Recently detected for the first time in Russia in 2016, the disease is of global significance. Root mat disease in tomato was first confirmed in the UK in 2000, but first observed in 1999 in plants propagated in the Netherlands. The disease is caused by the bacterium *Rhizobium radiobacter* (previously *Agrobacterium* bv. 1) containing a root inducing *Rhizobium cucumopine* (pRi) plasmid. Part of this plasmid (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 pRi plasmid to be contained within a number of other bacteria, including members of the genera *Ochrobactrum*, *Rhizobium* and *Sinorhizobium,* which can induce root mat symptoms in both tomato and cucumber. 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.

The most common and recognisable symptom of root mat disease in tomato is extensive root mat proliferation within the propagation cube. This 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 and irrigation issues. Excessive root growth may in extreme cases lead to swelling and distortion within the cube 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. Non-pathogenic strains of *R. radiobacter* are ubiquitous in soils, circulating liquid nutrient media and associated plant material.

A rapid molecular test developed by Fera Science Ltd., as part of this project, is now available which detects the presence of T-DNA from different 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 of 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.

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. In 2016 no grower reported slight symptoms, with all recording symptoms as either moderate or severe (10% or above cube coverage). Two growers who sourced their plant material from propagators in the UK in 2017 showed no symptoms of root mat disease. UK propagators are restricted in the quantity and range of products they can apply at this stage and may have more stringent hygiene practices. If propagation material arrives uninfected and site clean-up at crop turnaround is comprehensive, it is entirely possible that growers could eliminate the disease from their sites. Over two-thirds of growers questioned removed the plastic wrapper from slabs in order to reduce the impact of root mat disease. The efficacy of this method, which is yet to be scientifically confirmed, is due to be determined as part of this project on one site in 2018. 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.

Overall, the growers questioned considered the impacts of irrigation, subsequent drainage and substrate aeration important. There is also a 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.

Work performed in 2017 will quantify 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 will utilise the qPCR molecular test developed by Fera to confirm infection, including at times at plant arrival where plants may appear symptomless.

Laboratory work performed by Fera will also determine the most effective biocides use at crop turnaround on pure cultures and biofilms of *R. radiobacter.*

Materials and methods

Materials and methods for Objectives 1-3 are located in the PE 029 2016 report.

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

During 2017 two products, Carbon Gold Biology Blend (Carbon Gold, Product code: GRO203) and Vitix (Koppert, Product code: 102030) were examined to determine their effect on reducing tomato root mat incidence and severity in three commercial trials, Trials A, B and C. These two products were chosen based on performance of the same or similar products as part of the work carried out in Objective 3 during 2016. The trials consisted of five treatments and a double untreated control in a fully randomised blocked structure consisting of 42 plots spread over six replicates. There were 80 assessed cubes per plot in Trials A and between 36 and 108 cubes per plot in trials B and C. Errors in the number of treated plants at the propagator stage were responsible for the differences in cubes per plot included in this work. 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. Both ANOVA and Regression statistical tests were used in the analysis of root mat disease incidence, severity and qPCR data.

Treatments – during propagation

Three trials were established at two large commercial sites, Sites 1 and 2, in the South and East of England respectively. Both sites sourced their plant material from large, well-known Dutch propagators. These two propagators applied both Carbon Gold and Vitix to the plug plants on behalf of the project. One of the two propagators is good seed and plant practices (GSPP) certified, whilst the other follows GSPP guidelines, but is not certified. Tomato plug plants were treated according to manufacturer recommendations (Table 5) with a large quantity of plants (around 10, 000) treated for each trial. During propagation, each block of treated and untreated plants were kept separate with no biological or microbial products applied. Conventional fungicide use was also minimised, only used if absolutely necessary. Seed samples and an ebb-flood solution sample were requested from the first and last watering to be tested by Fera Science Ltd. for the presence of the Ri plasmid.

Treatments – at and after planting

Carbon Gold was applied at planting to determine if this enhanced any effect from propagation. The nature of Carbon Gold makes it impractical to do any subsequent applications after planting. Vitix was examined as a single application during propagation and as a series of drench applications after planting, and as a combined propagation and post-planting treatment. Vitix was reapplied every eight weeks from planting until November.

Table 5. A summary of the timing and doses of the treatments used in the three commercial trials in 2017

Trt	Treatment	Timing	Dose
1	Untreated	-	-
2	Untreated	-	-
3	Carbon Gold	Propagation	5 g, dusted around plug
4	Carbon Gold	Propagation	5 g, dusted around plug
		and at planting	55 g between cube and slab
5	Vitix	Propagation	1.5 g/m^2
6	Vitix	In-crop (repeated every 8 weeks)	3 g/100 plants
7	Vitix	Propagation & in-crop (repeated every 8 weeks)	1.5 g/m^2 3g /100 plants

Crop details

Trial A in the South of England consisted of the cherry tomato cv. Piccolo grafted onto slightly generative Optifort rootstock. Plants arrived on the 16th of January 2017 and were grown on Botanicoir propagation cubes (product code: GC01_101006_NW, Figure 7). These were then treated and placed on new, unused Botanicoir Breeze slabs (product code BR01_1001511_W) in a glasshouse compartment with a history of root mat disease. Each plot consisted of one entire row, with one plant per cube pinched to allow two heads to develop per plant.

Trials B and C were both located in the East of England at the same site. These trials were structured in a different way to Trial A. In both trials, rockwool propagation cubes (Grodan Plantop delta (Figure 8) were used which were placed onto coir slabs on the 3rd of March 2017. All plants used at this site were ungrafted. Due to the absence of rootstock in both of these trials, two plants were planted per cube, rather than having one plant which is later split to form two heads. Trial B consisted of cv. Piccolo, with once-used coir slabs, which had a history of the disease (from the 2016 season) and consequently contained some old matted roots. Second and even third-hand slabs have been used at this site previously, with the grower claiming better yields in plants grown in older slabs than new slabs. However, the grower had never before used second-hand slabs with a history of the disease. The best and most uniform slabs were chosen for use in the trial area, with those of poorer quality removed. Each plot consisted of 80 propagation cubes (40 slabs), containing two plants per cube.

Figures 7 & 8. Examples of the coir propagation cubes used in Trial A at Site 1 (left) and the rockwool propagation cubes used at Site 2 in Trials B and C (right)

Trial C comprised an ungrafted, baby plum variety cv. Funtelle. Cubes containing two plants were placed on brand new, unused coir slabs. The majority of the coir slabs used in the trial were Botanicoir Breeze (Product code: BR01_1001511_W), with four rows of Milleniumsoils (product code: 407) and six rows of Riococo slabs (Product code: unknown) also used. In all three commercial trials, the plastic wrapper of the surface of the slabs were removed as it is believed by many growers to reduce symptom severity, aiding aeration and light interception.

In all trials, the two slabs (comprising 4 cubes) at the end of each row were not assessed to avoid edge effects. In order to maximise the number of treated plants available in each treatment, each plot of Trial C comprised one and a half rows, a total of 108 cubes per plot. In Trial C, the numbers of treated plants delivered did not correspond with those originally ordered; the trial was therefore modified to compensate for these differences. Trial plans for each of the three trials are shown Appendix 1 (Trials A, B and C).

Carbon Gold, an insoluble form of biochar, was applied in-crop between the cubes and the slabs following wetting up, with no subsequent treatment throughout the duration of the trial. Vitix, a water soluble treatment, was applied following planting by hand-drenching directly over the plant roots. It was necessary to apply Vitix in this way due to the randomised nature of the trial. If Vitix was applied through the irrigation system, valve installation would have been needed to prevent mistreatment, an impractical request to ask of the host nurseries. Irrigation treatment would also have required the purging of the system to eliminate all trace of Vitix from the lines following each treatment. There is evidence that levels of dissolved oxygen in the irrigation system may play a role in disease management and continual purging of the system may cloud the results gained in this project. Some Dutch tomato growers are reported to flush standing water from pipework before applying the first irrigation of the day to plants in order to avoid using water with a reduced oxygen content (P. Bouwens, Grodan, pers. Comm.). Unlike Carbon Gold, Vitix, applied as per label, required repeat treatment every eight weeks throughout the growing season in plants grown in a coir substrate. Where Vitix is applied to rockwool application is repeated every four weeks

Pesticide application, including microbial products, via the irrigation lines was avoided where possible. Any other measures in place on growers' sites to control root mat in tomato were also recorded. A temperature and humidity logger was also placed in each trial to record environmental conditions from plant arrival. Temperature and humidity data for Trials A – C for the entire growing season are located in Appendix 5.

Assessments

Each trial was 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 root mat disease incidence and severity at each assessment date. At plant arrival a preliminary visual assessment of plant vigour, and the occurrence of tomato root mat (number of plants infected) was recorded.

In the case of Trial B, an additional visual slab assessment was performed to determine the coir slab quality and the quantity of old root mat present in the second year bags prior to planting (Table 6). This provided the option to discard any unsuitable slabs from future assessment and analysis. Unsuitable slabs were considered as those which exhibited severe root matting in patches, or over the entirety of the slab, and would not allow a crop to grow to commercial standard. This was not necessary in Trials A and C which used new coir slabs.

Table 6. Assessment criteria for the second-year infected slabs used in Trial B at Site 1 – March 2017

Slab quality	
Score	
0	Unusable, significant damage / quality issues
1	Very poor quality
2	Poor quality
3	New / replaced slab
Old root mat presence	
Score	
0	Unusable, severe old root mat presence
1	Moderate old root mat presence, usable
2	Minor old root mat present, usable
3	New / replaced slab, no root mat present

Root mat incidence and severity

Each trial was visited shortly after the first visual symptoms (incidence) of root mat disease had developed, at mid-cropping and again towards the end-of-cropping (dates for each assessment are located in Table 7.)

Table 7. The dates of the first-symptom, mid-crop and end-of-cropping assessments in trials A, B and C.

Assessment	Site 1 Trial A	Site 2 Trial B	Site 2 Trial C
First-symptom	27/04/2017	28/06/2017	28/06/2017
Mid-crop	31/07/2018	31/08/2017	31/08/2017
End-of-cropping	07/11/2017	14/11/2017	14/11/2017

All cubes were assessed for the incidence of the disease and the severity based on the scoring criteria in Table 7. Early root mat infection symptoms can be subtle and difficult to see on rockwool cubes where the surface can appear infected. For early assessments, cubes which showed ‘possible’ root mat infection were scored as ‘potentially’ infected.

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

At the final assessment for all trials, and in addition to tomato root mat disease incidence and severity, the number of dead plants per plot were recorded. Due to the length of each tomato stem, and the design of Trial B it was necessary to determine dead / missing plant number per plot by assessing the base of the plant stem as it entered the propagation cube.

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. These ‘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 3.

Root sampling for testing at Fera Science Ltd.

At the first-symptom and end-of-cropping assessments, root samples were collected from Trials A, B and C from all treatments. These samples were sent to Fera Science Ltd. for the detection of the Ri plasmid T-DNA using the rapid qPCR test developed in Objective 2 during 2016. Root samples were tested in addition to the seed and water samples tested from the propagators. On all sampling occasions, nitrile gloves were used and disinfected or changed between sampling different treatments to minimise the risk of cross-contamination.

To determine if root mat disease was present on asymptomatic young plants at arrival on site, a small pinch of roots (1-2 cm) was taken from 200 plants from the three propagation treatments, untreated, Carbon Gold and Vitix. Samples from each treatment were pooled into three separate bags and sent to Fera Science Ltd. for qPCR analysis.

At the first full in-crop 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. Root samples were bulked together to produce two bags per plot.

The root sampling procedure was repeated at the end-of-cropping assessment at the end-of-cropping for plots which had tested negative for the Ri plasmid T-DNA at the first in-crop assessment. This was performed in order to determine if root mat disease infection was now present in these previously uninfected plots. At this final assessment, tomato fruit were taken from plants which exhibited severe symptoms, from Site 1, for assessment of fruit and seed infection by molecular testing.

Objective 5 - To determine the efficacy of a selection of biocides used at crop turnaround in reduction of Rhizobium populations and Ri plasmid

Efficacy of different biocides was compared in the laboratory by two experimental methods:

Experiment 1: Treating aqueous suspensions of a pure culture of *R. radiobacter* (NCPPB 4062) with different concentrations of each biocide for different exposure times and measuring survival of the bacteria by sub-culturing on nutrient dextrose agar.

Experiment 2: Treating biofilms of *R. radiobacter* (NCPPB 4062) smeared onto segments of PVC dripper irrigation pipe by immersing in each biocide for different exposure times and measuring any growth of surviving bacteria during subsequent enrichment in 1A broth by pre- and post-enrichment qPCR analysis.

Four biocides were evaluated: Clorious$_2$ (Brenntag; CAS No. 100 49-04-4), Geosil (GeoSIL Pacific Limited; Cas No. 7722-84-1), Endosan3 (Endo Enterprises (UK) Ltd.; CAS No. 7722-84-1) and new formulation Domestos Extended Power (Unilever UK; CAS No. 7681-52-9/ 68955-55-5/1310-73-2). Samples of each product were provided with recommended doses from the UK manufacturers/distributors as shown in Table 8).

Table 8. The recommended doses and active ingredients of the four biocides tested during 2017

Biocide	**Active ingredient**	**Recommended dose**	
		Water treatment	**Shock treatment**
Clorious$_2$	Chlorine dioxide	0.75 ppm ClO_2	7.5ppm ClO_2
Geosil	Hydrogen peroxide and stabilized silver	50 ppm H_2O_2	500 ppm H_2O_2

Endosan3	Hydrogen peroxide and stabilized silver	50 ppm H_2O_2	500 ppm H_2O_2
Domestos	Sodium hypochlorite	1620 ppm NaOCl	1620 ppm NaOCl

Experiment 1

An aqueous suspension of *Rhizobium radiobacter* (NCPPB 4062) was prepared by washing overnight cultures grown on nutrient dextrose agar (Lelliott and Stead, 1987) and diluting to approximately 10^7 CFU per ml in sterile distilled water. The suspension was then aliquoted into 10 ml quantities. Each biocide was added to 3 replicated 10 ml quantities of the suspension to obtain concentrations of 10x, 5x, 1x, 0.5x and 0.1x of the recommended doses (as shown in Table 8). Residual concentrations of H_2O_2 and ClO_2 were verified using indicator strips (Endo Enterprises (UK) Ltd. Per 100 and Per 1000 and Precision Lab Inc., AZ, USA chlorine dioxide strips). Sterile distilled water was added as negative controls. After thorough vortex mixing, 0.1 ml subsamples were removed from each treated suspension after 0, 2, 5 and 10 min exposure to the biocide and spread onto nutrient dextrose agar. Growth of any surviving bacteria was then observed after incubation of the agar plates at 28°C for 48 hours. Growth was scored as:

- \- No regrowth of *R. radiobacter* after treatment
- +++ Full regrowth of *R. radiobacter* (10^7 CFU per ml) after treatment equivalent to water treated control
- ++ Reduced regrowth of *R. radiobacter* (10^4-10^6 CFU per ml) after treatment compared with water treated control
- \+ Reduced regrowth of *R. radiobacter* (10^2-10^4 CFU per ml) after treatment compared with water treated control
- (+) Reduced regrowth of *R. radiobacter* (10-10^2 CFU per ml) after treatment compared with water treated control

Experiment 2

Cultures of *R. radiobacter* (NCPPB 4062) were grown overnight on nutrient dextrose agar at 28°C. Segments of PVC dripper tubing (1 cm long) were coated in bacterial biofilm by dragging them through bacterial growth on the agar. Each biocide was prepared in replicated 20 ml quantities at the shock treatment concentrations (as shown in Table 8). Concentrations of ClO_2 and H_2O_2 were verified using indicator strips (Endo Enterprises (UK) Ltd. and

Precision Lab Inc., AZ, USA) before and after treatment. The contaminated tubing segments were submerged in each biocide, vortex mixed for 20 sec and then left for exposure times of 2, 5 or 10 minutes. Following exposure, they were air dried then each added to a tube containing 10ml 1A enrichment broth (Weller and Stead, 2002). After vortex mixing, 0.1 ml subsamples were removed, and cells were lysed by heating to 96°C for 5 minutes to expose bacterial DNA for PCR analysis. Enrichment of any remaining viable bacteria in the 1A broth was then performed for 72 hours at 26-28°C on a shaking incubator at 150 rpm. After enrichment, further 0.1 ml subsamples were removed, and bacterial DNA was exposed by lysing the cells by heating to 96°C for 5 minutes. Any bacterial growth during enrichment was then determined by performing qPCR TaqMan analysis on 1 µl of each of the exposed DNA samples. Growth was indicated by a decrease in critical threshold (Ct) values when pre-and post-enrichment samples were compared. Uninoculated enrichment buffer was used for negative controls. DNA obtained from a reference strain of *R. radiobacter* was used for positive controls.

Additional work

1. *Potential impacts of irrigation systems on the spread and management of tomato root mat disease*

The grower at Site 2 treated an additional block of cv. Funtelle with a higher rate of Vitix to that used in the neighbouring Trial C. This block was grown following the normal commercial standards used at this site. Following the analysis of the first-symptom assessment results, this area was assessed to observe if a greater rate of Vitix application had an enhanced effect on root mat control. The irrigation system in this area is unusual as it feeds both from the front and the back of the rows simultaneously meeting in the centre. Water destined for the back half of each row has considerably further to travel than water destined for the first half as it must be transported to the back of the rows first. Levels of dissolved oxygen decrease where water remains in irrigation systems for long periods of time and lower oxygen content is conducive to growth of *R. radiobacter*. This is potentially important at the start of the season where irrigation events are less frequent and water will remain in the system for longer. This may result in a greater quantity of *R. radiobacter* present in the water destined for the back half of each plot, which may lead to greater levels of infection in these areas, especially as younger plants are more susceptible to root mat infection. The spatial distribution of the incidence and severity of root mat disease was visually assessed and mapped to see if this theory is correct. The central six of the eight rows were assessed, with the first two slabs not assessed to avoid any edge effects. The trial was assessed at the same time as the mid-crop

and end-of-cropping assessments at Site 2, in July and November. A plan of this additionally treated area is located in Appendix 2.

As only one treatment rate of Vitix was applied and no untreated controls were present the outcomes from this additional work are anecdotal and would need to be confirmed via a scientifically robust trial in the future.

2. *Brown root rot assessment*

Severe cases of tomato root mat are often associated with an increase in secondary root infections. At the mid-crop assessment, the location of any brown roots was recorded in the trials to determine if any treatment reduced the incidence of brown roots compared to the untreated control.

Results

Results for Objectives 1-3 are located in the PE 029 2016 report.

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

qPCR analysis of approximately 100 Piccolo seeds sent to Fera for testing from the GSPP approved Dutch propagator supplying Site 1 (Trial A) tested negative for the Ri plasmid. A water sample taken during the final irrigation of the tomato plants also tested negative for the bacterium and pRi.

Seed and final irrigation water samples were also sent from the Dutch propagator supplying Site 2 (Trials B and C). The qPCR analysis revealed that the Piccolo seed (Trial B, only 10-15 seeds supplied) and Funtelle seed (Trial C, roughly 100 seeds), and the water samples all tested negative for *R. radiobacter* pRi. These results indicate that tomato root mat disease was not seed-borne in these trials. The absence of the Ri plasmid in the water samples tested suggest the propagators used, and the areas used to store the tomato plants for both sites, were free of the 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, so that the possibility of seed and / or water-borne infection cannot be completing discounted and more intensive future monitoring is recommended. No water sample was provided for testing from the first irrigation from either propagator.

Testing of composite treatment samples at plant arrival

For each of the three treatments at propagation, a small sample of Carbon Gold, Vitix and untreated plant roots were taken from 200 seedlings and bulked together into three composite samples per trial at arrival on the two commercial sites. In Trial A, the presence of the Ri plasmid DNA was detected via direct DNA extraction in samples taken from the untreated control, but not from root samples collected from the Carbon Gold or Vitix treated populations (Table 9). Positive results were also obtained following incubation of root extract in an enrichment medium. This indicates that viable bacteria containing the Ri plasmid was present in or on the roots of the control plants and grew to detectable levels after dilution in the enrichment medium and subsequent incubation. The sampling rate per treatment is sufficient to detect an infection level of 1.5% or more with a confidence of 95%.

Table 9. Site 1, Trial A - qPCR results of the three replicate composite root samples treated with Carbon Gold, Vitix and untreated plants at propagation sampled at plant arrival – January 2017

Treatment	Sample	Direct Extraction Cox CT (control)	Direct Extraction Ri CT	Pre-Enrichment Ri CT	Post-Enrichment Ri CT
Carbon Gold	A	**18.75**	40.00	40.00	40.00
	B	**19.28**	40.00	40.00	40.00
	C	**18.93**	40.00	40.00	40.00
	Average	**18.99**	40.00	40.00	40.00
Vitix	A	**18.71**	40.00	40.00	40.00
	B	**19.13**	40.00	40.00	40.00
	C	**18.66**	40.00	40.00	40.00
	Average	**18.83**	40.00	40.00	40.00
Untreated	A	**18.49**	**33.34**	40.00	**38.51**
	B	**18.39**	**37.82**	40.00	**33.92**
	C	**18.25**	40.00	40.00	**32.84**
	Average	**18.38**	**37.05**	40.00	**35.09**

Values in bold in results columns two and four indicate detection of the Ri plasmid at a low / average level of infection

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 also run and obtained a CT value of 18-19 which indicates successful DNA extraction and amplification from each sample.

qPCR analysis of composite root samples at Site 2, Trial B (Table 10) revealed contrasting results to that of Trial A. Results for Trial B show that no infection was detected in either the Carbon Gold, Vitix or untreated plants indicating the entire crop in Trial B arrived clean. Results for Trial C at Site 2 showed a low level of infection, similar to that seen in Trial A, however unexpectedly infection was seen in plants treated with Carbon Gold and Vitix at propagation but not seen in untreated plants. In this case, the internal positive control run gave CT values between 21 and 22 indicating successful extraction and amplification from each sample.

The same Dutch propagator produced the Piccolo plants for Trial B and Funtelle plants for Trial C. The fact that infection was detected in Funtelle plants at arrival suggests that infection could have occurred at the propagation stage. The qPCR tests were negative for cv. Piccolo root mat infection at arrival on site. This suggests that if *R. radiobacter* is present at the propagator used for Trials B and C it may be present sporadically. This would explain why infection was confirmed in the Funtelle and not the Piccolo crop. Alternatively differences in varietal susceptibility may exist between these two cultivars explaining the qPCR results seen. This is less likely however as effective varietal resistance against root mat disease has not been shown to exist and the Piccolo crop in Trial A, sourced by a separate propagator, and was shown to suffer infection at arrival at Site 1.

Table 10. Site 2, Trials B and C - qPCR results of the three replicate composite root samples treated with Carbon Gold, Vitix and untreated at propagation, sampled at plant arrival – March 2017

Trial B	**Piccolo**				
Treatment	Sample	Direct Extraction Cox CT (control)	Direct Extraction Ri CT	Pre-Enrichment Ri CT	Post-Enrichment Ri CT
Carbon	A	**21.64**	40.00	40.00	40.00
Gold	B	**21.48**	40.00	40.00	40.00
	C	**21.61**	40.00	40.00	40.00
	Average	**21.58**	40.00	40.00	40.00
Vitix	A	**21.53**	40.00	40.00	40.00
	B	**20.41**	40.00	40.00	40.00
	C	**21.42**	40.00	40.00	40.00
	Average	**21.12**	40.00	40.00	40.00
Untreated	A	**21.80**	40.00	40.00	40.00
	B	**21.99**	40.00	40.00	40.00
	C	**22.08**	40.00	40.00	40.00
	Average	**21.96**	40.00	40.00	40.00
Trial C	**Funtelle**				
Treatment	Sample	Direct Extraction Cox CT (control)	Direct Extraction Ri CT	Pre-Enrichment Ri CT	Post-Enrichment Ri CT
Carbon	A	**21.46**	**35.24**	40.00	40.00
Gold	B	**20.91**	**37.36**	40.00	40.00
	C	**21.31**	40.00	40.00	40.00
	Average	**21.23**	**37.53**	40.00	40.00
Vitix	A	**23.27**	**37.19**	40.00	40.00
	B	**21.56**	**36.04**	40.00	40.00
	C	**21.03**	**38.50**	40.00	40.00
	Average	**21.95**	**37.24**	40.00	40.00
Untreated	A	**21.59**	40.00	40.00	40.00
	B	**20.82**	40.00	40.00	40.00
	C	**21.67**	40.00	40.00	40.00
	Average	**21.36**	40.00	40.00	40.00

Values in bold in column two indicate detection of the Ri plasmid at a low / average level of infection

Preliminary assessments – at plant arrival

Root mat incidence and plant vigour were visually assessed at plant arrival. In all three trials no symptoms of root mat disease were observed. Plant vigour was assessed by the staff at Site 1 in January and was scored as healthy, with a medium vigour score of three (1 = very poor, 5 = perfect) in Trial A. This score was considered ideal, as strong vigorous plants at this very early time of year can increase the risk of Botrytis development. The tomato plants for both Trials B and C which arrived later in March were strong and healthy and were given the maximum score of five.

Trial B was placed out on once-used slabs with a history of the disease. Any slabs of particularly poor quality were not used in this trial. Slabs were scored based on their quality and the presence of previous years root mat. All slabs were visually assessed and all were deemed usable, with the vast majority showing only low levels of damage (as would be expected for a once-used slab). The level of damage throughout all the slabs was considered to be uniform and the quality sufficient to allow the trial to proceed. The level of root mat was also constant throughout the slabs with only minor root mat presence from the 2016 season.

Assessment 1: At first symptoms

Trial A - Plants arrived at Site 1 on the 16th of January 2017. The first root mat symptoms were detected in the glasshouse compartment containing this trial on April 27th. Root mat symptoms manifested later than expected having been recorded in other houses on Site 1 earlier. The first symptom assessment, including root collection for qPCR analysis was completed on the 3rd of May 2017

Disease incidence was very low at this first assessment. In total, 40 confirmed and 20 potential incidences of root mat disease were recorded on over 4000 cubes assessed. Interestingly, despite the low incidence, there were statistically significant differences between the incidence and severity score distribution of different treatments at this date.

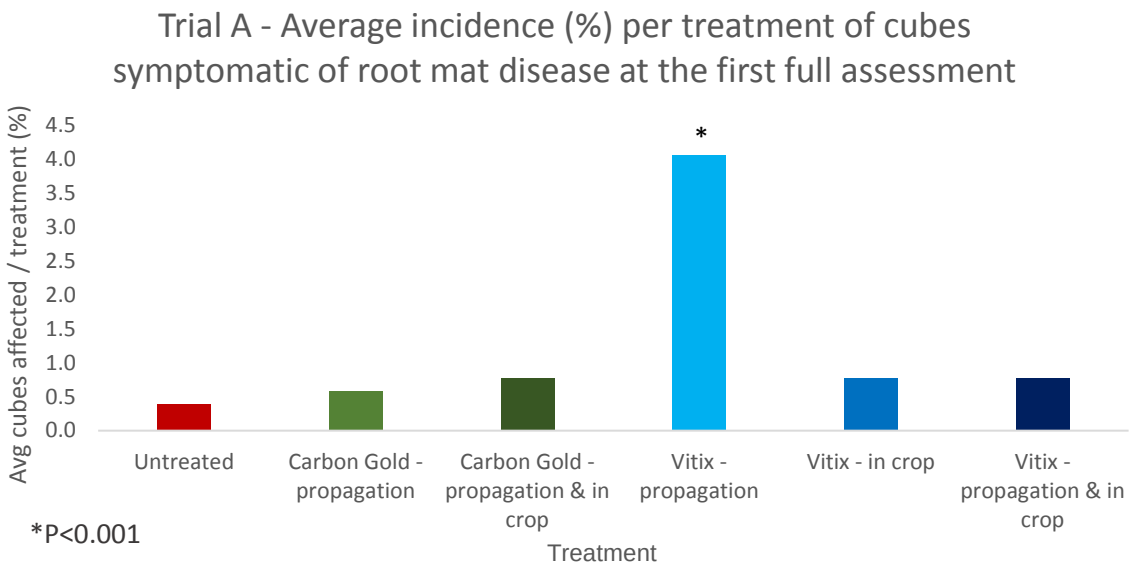


Figure 9 – Incidence (%) of root mat disease in treated and untreated plots in Trial A at the first full assessment – May 2017

* indicates a significant increase in disease incidence compared to the untreated control

Statistically highly significant results were found when the data was analysed using a Genstat regression analysis looking at the confirmed cases of root mat incidence, Figure 9. The same outcome was also recorded when the 20 potentially infected cubes were included in the analysis ($P<0.001$). No treatment was found to decrease the incidence of root mat disease compared to the untreated (0.4% cubes affected / plot). Cubes containing plants treated with Vitix at propagation alone showed a large increase in root mat incidence (4.1% of cubes affected with root mat disease, $P<0.001$). No significant differences were seen between other treatments and the untreated control at this time.

Table 11 shows the distribution of each of the severity scores in the form of a heat map based on the average amount (%) of the plants affected per treatment. Green cells correspond to low numbers of cubes exhibiting a certain severity score. Yellow through orange to red correspond to more frequent incidences of each score respectively. Despite the low number of severity scores at this assessment, Vitix treated in propagation alone showed a much higher proportion of high scores than the other treatments. The presence of these high scores (a score of 6 corresponding to above 50% of slab surface affected) in tomato plants treated with Vitix at propagation alone suggests that the disease was likely visible before April 27th. Figure 10 shows an example of a severe infection of a cube containing a tomato plant treated with Vitix in propagation alone.

Table 11 – Distribution of severity scores (based on the percent of plants affected) for the five treatments and untreated control in Trial A at the first symptom assessment – May 2017

Treatment	Severity score					
	1	2	3	4	5	6
Untreated (averaged)	0.1	0.0	0.0	0.1	0.2	0.0
Carbon Gold - propagation	0.2	0.0	0.0	0.0	0.2	0.2
Carbon Gold - in propagation & in crop	0.2	0.2	0.0	0.0	0.4	0.0
Vitix - propagation	0.2	0.4	0.2	1.0	1.2	1.2
Vitix - in crop	0.0	0.2	0.0	0.2	0.2	0.2
Vitix - propagation & in crop	0.4	0.0	0.0	0.0	0.2	0.2

Progression from green to red corresponds to larger numbers of cubes affected per treatment for each severity score

Figure 10 - A severe infection (severity score of 6) seen in Trial A in a plant treated with Vitix at propagation at the first symptom assessment on May 3rd 2017. Note that even at this early roots have spread from the cube surface to the slab

Tomato root mat can cause crop losses due to irrigation problems which arise from more severe infections allowing water to run off the cube / slab rather than be absorbed. Although not ideal, plants suffering a lesser extent of root mat coverage are better able to be managed, however more extreme symptoms will most likely develop over time. The amount (%) of cubes per treatment with a severity score greater than three, corresponding to cube surface coverage above 10%, was analysed using Genstat regression analysis. Again statistically highly significant results were found, Vitix applied at propagation alone increased the percent of cubes with surface root coverage above 10% increasing from 0.3% in the untreated to

3.3% (Figure 11, P<0.001). No other significant differences were seen between any of the other treatments and the untreated control at this assessment.

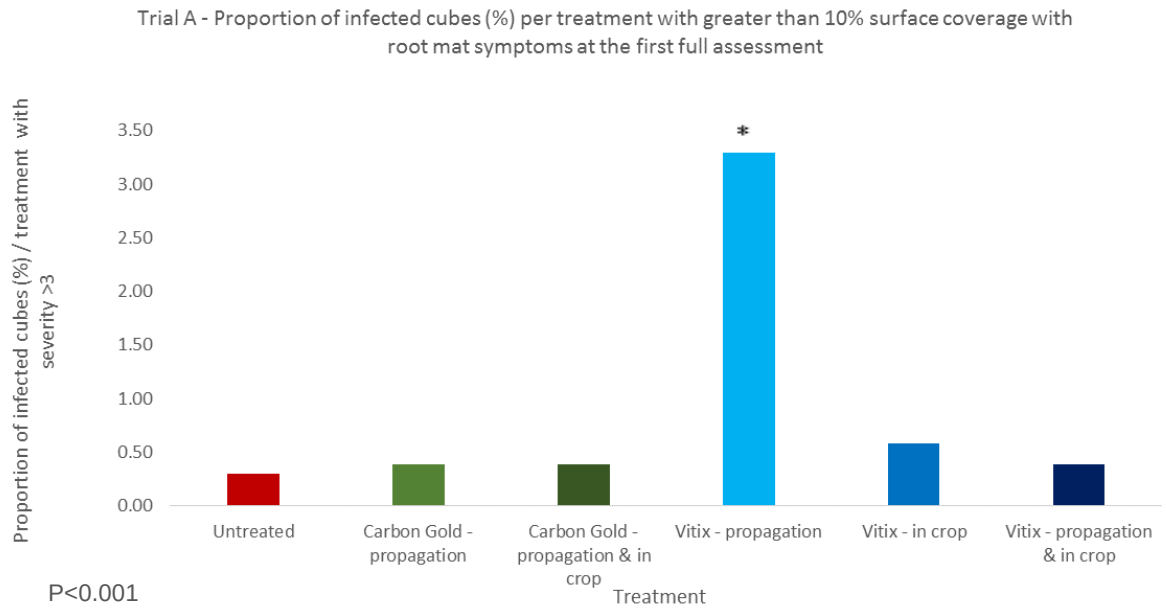


Figure 11 – The proportion of cubes from each treatment with root mat severity coverage greater than 10% in Trial A at the first symptom assessment – May 2017
* indicates a significant increase in disease incidence compared to the untreated control

At the first assessment, 25 out of the 42 trial plots showed no visible symptoms of root mat disease. Infection was seen in at least two plots per treatment, demonstrating that no treatment provides complete control. The number of visually infected plots for each treatment for the six replicates at the first symptom assessment is found in Table 12. Interestingly, the untreated plants showed the fewest infected plots with only two out of twelve untreated plots visibly infected (double untreated control). Plants treated with Vitix at propagation alone saw the greatest number of plots infected; four out of six, which corresponds with the incidence and severity results seen at this assessment. The fact that root mat infection was not directly detected in root samples from the two sets of treated plants at plant arrival suggests the disease may have originated from a pre-existing inoculum source on site, or was at such low incidence that the sampling of 200 plants for the qPCR test was unable to detect it.

Table 12. Number of plots for each of the treatments displaying visual symptoms of root mat disease in Trial A – May 2017

Treatment	No. of plots per treatment	No. of infected plots
Untreated (12 plots)	12	2
Carbon Gold - propagation	6	3
Carbon Gold - in crop	6	2
Vitix - propagation	6	4
Vitix - in crop	6	3
Vitix - propagation & in crop	6	3

Plants were delivered to Trials B and C on the 3rd of March 2017, several weeks after the planting date in Trial A. Due to this time difference the initial detection of root mat symptoms was seen later in Trial B on the 6th of June 2017. The intervals between planting and the first symptoms being observed was 14, 13 and 16 weeks in Trials A, B and C respectively. Trials B and C were assessed when symptoms had developed in both trials, on the 28th of June 2017. Root samples from asymptomatic and symptomatic plants were collected and sent to Fera Science Ltd. for qPCR analysis. Due to errors in treatment application in Trial B, data from plots 19 and 40 was excluded from all statistical analysis.

At the first-symptom assessment of Trial B, 483 confirmed cases of tomato root mat were recorded, with 25 potentially infected cubes in a trial of over 3000 cubes (cv. Piccolo). Incidence levels in the untreated plants were much higher than those seen in the untreated plants in Trial A (16.9% compared to 0.4% respectively). No differences in incidence were seen between any of the treatments and the untreated at this assessment (Figure 12, P=0.095). Unlike the first symptom assessment in Trial A, every plot in Trial B showed some visible degree of infection of tomato root mat disease.

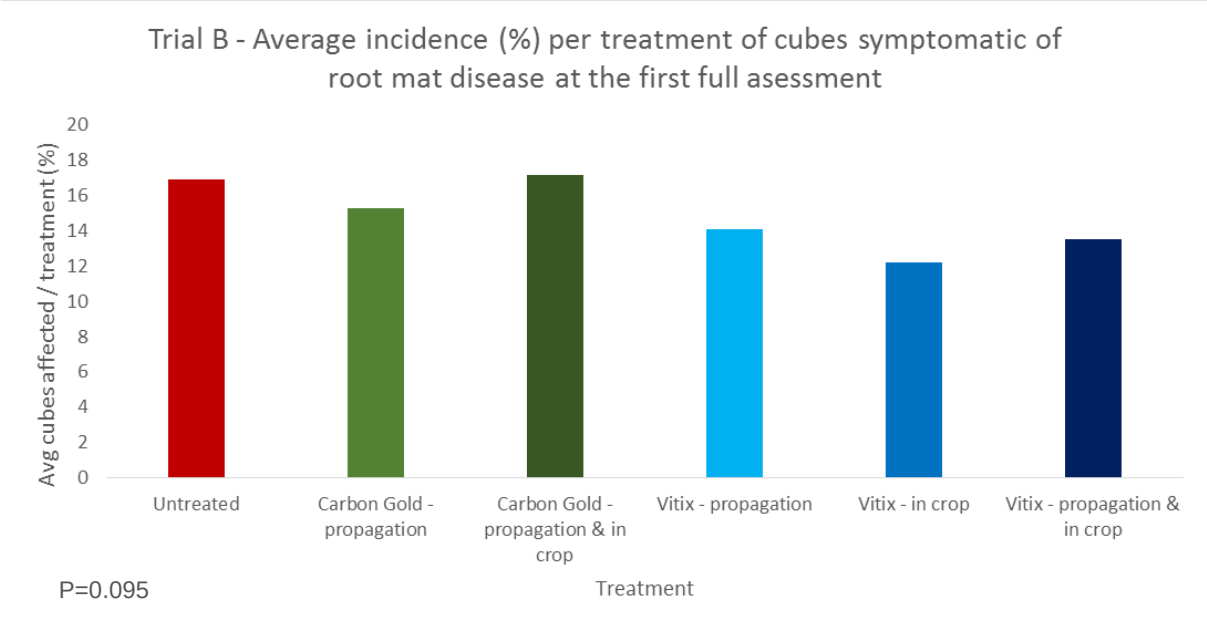


Figure 12 - Incidence (%) of root mat disease in treated and untreated plants in Trial B at the first symptom assessment, no infection was detected in plants at arrival on this nursery. Rockwool cubes were planted onto once-used coir slabs with a history of root mat disease – June 2017

The treatment severity scores for Trial B are shown in Table 13. Despite a much larger quantity of infected cubes, the distribution of severity scores is much more skewed to the low end compared to the results seen in Trial A. Vitix treated at propagation alone did not result in a much larger incidence / severity of root mat disease in this trial. No statistical differences were found between the untreated and any treatment in the proportion of scores above 10% of cube affected (P=0.480).

Table 13. Distribution of severity scores (based on the percent of plants affected) of the five treatments and untreated control in Trial B at the first-symptom assessment – June 2017

Treatment	Severity score					
	1	2	3	4	5	6
Untreated (average)	13.0	3.4	0.3	0.0	0.0	0.2
Carbon Gold - propagation	11.4	3.0	0.6	0.2	0.0	0.0
Carbon Gold - in propagation & in crop	13.2	3.3	0.7	0.0	0.0	0.0
Vitix - propagation	10.2	3.7	0.2	0.0	0.0	0.0

Vitix - in crop	11.2	1.0	0.0	0.0	0.0	0.0
Vitix - propagation & in crop	11.2	1.1	0.9	0.0	0.3	0.0

Progression from green to red corresponds to larger numbers of cubes affected per treatment for each severity score

The first-symptom assessment of Trial C (cv. Funtelle), on new slabs, showed a considerably lower incidence of tomato root mat disease compared to that in Trial B, cv. Piccolo on once-used slabs, which arrived at the same time and was located in a neighbouring glasshouse. In total only 50 confirmed incidences of root mat disease were recorded, with a further 50 potentials observed in a trial of 2800 cubes. Statistical analysis revealed no differences in root mat incidence between treated and untreated plants in Trial C at this time (Figure 13, P=0.98)

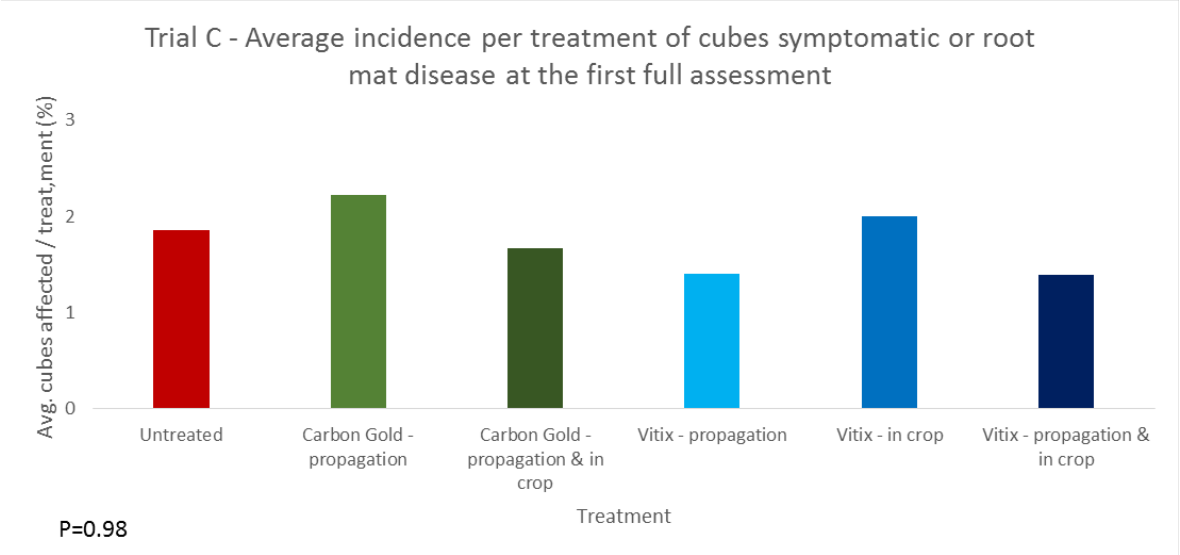


Figure 13. Incidence (%) of root mat disease in treated and untreated plots in Trial C at the first-symptom assessment – June 2017. Rockwool cubes were planted onto new coir slabs.

Treatment severity scores for Trial C are shown in Table 14. Despite a few higher scores seen in the untreated plots, severity scores were generally low with less than 2. 5% of any treatment symptomatic of the disease. No statistical differences were found in the proportion of scores above 10% cube coverage (P=0.081).

Table 14. Distribution of severity scores (based on the percent of plants affected) of the five treatments and untreated control in Trial C at the first symptom assessment – June 2017

Treatment	Severity score

	1	2	3	4	5	6
Untreated (average)	0.4	0.6	0.2	0.2	0.3	0.2
Carbon Gold - propagation	1.7	0.0	0.0	0.6	0.0	0.0
Carbon Gold - in propagation & in crop	0.0	1.1	0.0	0.6	0.0	0.0
Vitix - propagation	0.6	1.1	0.0	0.0	0.0	0.0
Vitix - in crop	1.1	0.9	0.0	0.0	0.0	0.0
Vitix - propagation & in crop	0.5	0.0	0.0	0.9	0.0	0.0

Progression from green to red corresponds to larger numbers of cubes affected per treatment for each severity score.

At this assessment, 20 of 42 plots were symptomatic of root mat disease, however infection was present in all treatments (Table 15). Carbon Gold treated in propagation alone had the fewest plots visibly symptomatic of tomato root mat disease. Vitix applied in crop alone and the untreated had the largest proportion of plots symptomatic of root mat, 4 and 4.5 (averaged over 6 plots) respectively.

Table 15. The number of plots containing visibly infected cubes at the first symptom assessment, Trial C – June 2017

Treatment	No. of plots per treatment	No. of infected plots
Untreated (12 plots)	12	9
Carbon Gold - propagation	6	1
Carbon Gold - in propagation & crop	6	2
Vitix - propagation	6	2
Vitix - in crop	6	4
Vitix - propagation & crop	6	2

Heat maps for Trials A, B and C have been produced for the first and last assessments in order to visualise the spatial and temporal spread of root mat disease at these sites. These are located in Appendix 3.

Assessment 1: Root sampling qPCR results

Trial A, Piccolo - qPCR analysis of bulked symptomatic and asymptomatic root samples revealed that despite 25 plots in Trial A being asymptomatic of root mat disease, the disease was actually present across all five treatments and in all but two plots. These were plots 4 and 15, Carbon Gold applied both at propagation and crop, and untreated respectively. Of the asymptomatic samples collected from each plot, 39 out of 42 were shown to contain infection. It is possible that if a greater quantity of roots were sampled that the disease may have been detected in all plots. Table 16 shows the qPCR results for the asymptomatic and symptomatic root samples. In all three trials, roots were tested using both a direct extraction as well as pre- and post-enrichment methods. Values in bold indicate infected samples, whilst missing values are due to no symptomatic cubes available from which to sample infected roots. The qPCR results samples taken at plant arrival in Trial A concluded that no infection was present in the young plants treated with Carbon Gold and Vitix. Confirmation of disease presence in every treatment 14 weeks later indicates the disease likely spread from a pre-existing inoculum source on site e.g. the irrigation system, or that plants were infected at arrival but at levels too low for the qPCR assay to detect.

Table 16 – qPCR results from asymptomatic and symptomatic bulked root samples taken at the first symptoms assessment, Trial A – May 2017

Plot	Treatment	Asymptomatic root samples			Symptomatic root samples		
		Direct Extraction	Pre-Enrichment	Post-Enrichment	Direct Extraction	Pre-Enrichment	Post-Enrichment
1	6	40.00	40.00	40.00	**20.80**	**31.38**	**23.51**
2	3	**36.75**	40.00	40.00	**27.07**	**35.22**	**23.19**
3	2	**27.79**	**37.84**	**28.82**	-	-	-
***4**	4	40.00	40.00	40.00	-	-	-
5	1	**33.01**	40.00	40.00	**32.04**	40.00	**30.72**
6	5	**20.73**	**31.73**	**24.08**	**19.38**	**27.39**	**23.88**
7	7	**28.19**	40.00	**30.89**	-	-	-
8	3	**30.17**	40.00	40.00	**22.58**	**29.75**	**25.61**
9	2	**29.74**	40.00	**38.52**	**28.84**	**38.02**	**32.98**
10	5	**28.37**	**38.53**	**31.79**	-	-	-
11	7	**23.93**	**34.84**	**22.12**	-	-	-
12	6	**33.31**	40.00	**38.31**	**34.29**	40.00	**35.67**
13	4	**28.47**	**38.42**	**35.92**	**33.93**	40.00	**35.20**
14	1	**31.39**	40.00	**30.78**	-	-	-
15*	2	40.00	40.00	40.00	-	-	-
16	5	**28.40**	40.00	**27.81**	**18.94**	**27.88**	**28.63**
17	1	**30.62**	**38.46**	**31.27**	**20.17**	**28.05**	**26.34**

18	3	**34.15**	40.00	**34.10**	**21.01**	**29.76**	**26.18**
19	7	**34.49**	40.00	**38.30**	**20.45**	**28.32**	**23.50**
20	4	**29.27**	40.00	**32.50**	-	-	-
21	6	**25.45**	**34.14**	**34.50**	-	-	-
22	1	**31.64**	40.00	**33.83**	**28.70**	**37.93**	**28.31**
23	3	**30.53**	40.00	**31.92**	-	-	-
24	6	**23.65**	**34.94**	**31.46**	**19.29**	**29.43**	**25.72**
25	4	**31.87**	40.00	40.00	-	-	-
26	7	**29.52**	**36.19**	**31.71**	**20.45**	**28.72**	**24.02**
27	5	**25.05**	**33.30**	**32.19**	-	-	-
28	2	**33.93**	40.00	40.00	-	-	-
29	4	**31.20**	40.00	40.00	**22.20**	**29.92**	**30.47**
30	2	**25.31**	**34.94**	**23.52**	-	-	-
31	1	**36.12**	40.00	**34.04**	-	-	-
32	7	**27.39**	**36.44**	**29.81**	-	-	-
33	3	**25.15**	**34.70**	**32.10**	-	-	-
34	2	**24.96**	**34.18**	**28.57**	**19.84**	**30.18**	**27.95**
35	3	**27.22**	**33.75**	**26.26**	-	-	-
36	5	**30.44**	40.00	**35.00**	**21.25**	**30.42**	**30.30**
37	1	**29.65**	**38.11**	**34.22**	**27.06**	**35.35**	**30.45**
38	4	**36.65**	40.00	40.00	**22.43**	**30.34**	**28.04**
39	7	**31.74**	40.00	**29.17**	**20.71**	**29.65**	**24.10**
40	6	**33.42**	40.00	**31.97**	**19.62**	**29.76**	**24.61**
41	3	**25.57**	**34.29**	**30.18**	-	-	-
42	2	**32.21**	40.00	**32.98**	-	-	-
Positive control		16.93	16.52	16.48	18.41	17.09	17.09
Negative control		40.00	40.00	40.00	40.00	40.00	40.00
Mastermix		40.00	40.00	40.00	40.00	40.00	40.00

Bold values - Plots which have tested positive for root mat disease

- - Missing samples due to no symptomatic roots available to sample

* - Plots which tested negative for root mat disease

Trial B, Piccolo - The qPCR results of root samples collected at the first symptoms assessment show that every plot is infected regardless of treatment (Table 17). The disease was found to be present in plant roots in all plots regardless of whether asymptomatic or symptomatic roots were tested or the method of detection used. Testing at plant arrival at Site 2 revealed no infection in Trial B in either treated or untreated plants. It is therefore probable that the disease originated on site in Trial B. As these plants were grown in unsterlized once-used slabs with a history of root mat disease it is likely the slabs themselves were the *R. radiobacter* and Ri plasmid source. Other varieties grown at this site in once-used slabs also

exhibited greater levels of infection compared to those grown in new slabs providing further evidence for this theory.

Table 17- qPCR results from asymptomatic and symptomatic bulked root samples taken at the first symptoms assessment, Trial B – June 2017

Plot	Treatment	Asymptomatic root samples			Symptomatic root samples		
		Direct Extraction	Pre-Enrichment	Post-Enrichment	Direct Extraction	Pre-Enrichment	Post-Enrichment
1	2	**24.88**	**33.46**	**27.44**	**24.25**	**33.63**	**22.87**
2	4	**24.86**	**34.90**	**27.58**	**23.12**	**31.91**	**24.43**
3	3	**24.92**	**34.04**	**29.06**	**21.98**	**31.81**	**25.53**
4	6	**25.99**	**36.11**	**29.29**	**25.81**	**33.75**	**25.93**
5	7	**27.30**	**34.13**	**24.64**	**24.08**	**33.88**	**30.92**
6	5	**28.28**	**35.03**	**29.53**	**25.04**	**32.28**	**27.62**
7	1	**28.12**	**36.66**	**32.40**	**24.00**	**32.99**	**27.26**
8	1	**23.95**	**33.72**	**26.76**	**23.84**	**33.18**	**27.66**
9	5	**27.05**	**34.20**	**28.13**	**25.44**	**32.89**	**23.16**
10	6	**25.98**	**34.43**	**25.42**	**24.60**	**32.70**	**23.74**
11	7	**27.95**	**36.23**	**25.81**	**23.46**	**32.47**	**24.85**
12	3	**29.77**	**37.15**	**22.59**	**24.69**	**31.23**	**25.21**
13	2	**30.83**	**36.13**	**26.10**	**23.46**	**33.07**	**27.14**
14	4	**29.38**	**36.04**	**26.46**	**24.02**	**33.46**	**26.50**
15	1	**28.86**	**36.09**	**30.46**	**26.28**	**32.86**	**27.07**
16	6	**27.51**	**38.84**	**24.04**	**24.72**	**32.21**	**26.19**
17	5	**25.23**	**34.58**	**24.46**	**24.31**	**32.30**	**25.64**
18	2	**26.72**	**35.01**	**27.55**	**26.03**	**33.17**	**26.79**
19	7	**27.79**	**34.41**	**24.10**	**23.31**	**30.80**	**31.09**
20	4	**27.00**	**33.30**	**24.53**	**24.69**	**33.09**	**22.46**
21	3	**25.82**	**34.19**	**25.73**	**23.21**	**31.42**	**26.04**
22	3	**25.85**	**34.54**	**26.60**	**26.90**	**35.44**	**24.64**
23	6	**29.51**	**31.80**	**23.62**	**26.00**	**33.48**	**24.09**
24	5	**25.60**	**32.66**	**28.13**	**26.70**	**36.38**	**28.42**
25	7	**26.81**	**33.04**	**23.73**	**24.93**	**34.96**	**22.69**
26	1	**26.67**	**34.50**	**28.23**	**29.03**	**38.95**	**26.93**
27	2	**27.31**	**36.61**	**26.64**	**29.26**	**35.82**	**23.97**
28	4	**26.43**	**34.68**	**23.59**	**25.89**	**35.21**	**25.12**
29	6	**26.93**	**35.47**	**26.53**	**27.10**	**36.23**	**21.69**
30	4	**28.25**	**36.63**	**23.73**	**26.77**	**35.01**	**27.06**
31	3	**28.09**	**35.57**	**24.19**	**23.84**	**35.50**	**24.79**
32	5	**27.18**	**34.45**	**20.32**	**25.88**	40.00	**21.30**
33	1	**27.17**	**34.79**	**21.69**	**26.90**	**34.83**	**22.06**
34	7	**25.94**	**35.95**	**22.59**	**26.92**	**34.07**	**20.79**
35	2	**28.91**	**35.37**	**22.41**	**26.05**	**34.02**	**22.10**

36	7	**30.98**	40.00	**27.08**	**28.85**	**35.07**	**26.32**
37	3	**26.00**	**33.97**	**23.28**	**28.21**	**38.02**	**21.51**
38	2	**27.15**	**35.12**	**27.04**	**27.20**	**35.96**	**23.29**
39	1	**26.25**	**35.34**	**23.03**	**27.07**	**33.66**	**23.16**
40	6	**27.51**	**33.99**	**20.83**	**26.92**	**36.17**	**23.81**
41	4	**27.01**	**35.65**	**24.33**	**26.37**	**33.91**	**24.68**
42	5	**26.82**	**36.17**	**23.33**	**25.62**	**34.96**	**22.79**
Positive control		31.84	29.97	19.82	31.84	29.97	19.92
Negative control		40.0	40.0	40.0	40.0	40.0	40.0
Mastermix		40.0	40.0	40.0	40.0	40.0	40.0

Bold values - Plots which have tested positive for root mat disease

Trial C, Funtelle – All bulked samples of asymptomatic and symptomatic roots tested positive for root mat disease by direct extraction or post-enrichment qPCR testing except for plot 25, Vitix treated at both propagation and in crop (Table 18). No direct extraction was performed on the symptomatic root samples as it was unnecessary due to confirmation of the disease presence via enrichment. Untreated plants tested negative for the disease at plant arrival but tested positive for root mat disease at this assessment.

Table 18- qPCR results from asymptomatic and symptomatic bulked root samples taken at the first symptoms assessment, Trial C – June 2017

Plot	Treatment	Asymptomatic root samples			Symptomatic root samples		
		Direct Extraction	Pre-Enrichment	Post-Enrichment	Direct Extraction	Pre-Enrichment	Post-Enrichment
1	6	**32.46**	**37.37**	**32.43**		**37.48**	**33.90**
2	7	**29.74**	40.00	**29.02**	**-**	**-**	**-**
3	2	**34.94**	40.00	**31.36**	**-**	**30.48**	**23.22**
4	3	40.00	40.00	**36.70**	**-**	**-**	**-**
5	1	40.00	40.00	**38.81**	**-**	**35.89**	**25.39**
6	4	**32.33**	40.00	**33.69**	**-**	**-**	**-**
7	5	**26.97**	**34.76**	**24.91**	**-**	**34.39**	**23.21**
8	6	**33.56**	40.00	**31.62**	**-**	**30.07**	**24.96**
9	1	**26.14**	**36.26**	**24.75**	**-**	**27.81**	**24.63**
10	2	**28.37**	**35.00**	**24.70**	**-**	**28.90**	**24.03**
11	5	**28.28**	**35.51**	**28.88**	**-**	**34.12**	**22.72**
12	3	**32.84**	40.00	**30.41**	**-**	**-**	**-**
13	7	**32.10**	40.00	**30.17**	**-**	**33.85**	**23.28**
14	4	**36.96**	40.00	**35.86**	**-**	**30.43**	**27.94**
15	3	**30.75**	40.00	**38.90**	**-**	**-**	**-**
16	6	**29.75**	**37.31**	**30.71**	**-**	**30.21**	**22.16**
17	1	**31.75**	40.00	**35.98**	**-**	**30.58**	**22.74**
18	5	**34.87**	40.00	**31.46**	**-**	**31.13**	**22.78**
19	2	**28.82**	**35.64**	**29.56**	**-**	**31.87**	**25.86**

20	4	**30.03**	40.00	**27.89**	**-**	**-**	**-**
21	7	**24.74**	**33.03**	**26.42**	**-**	**35.32**	**30.94**
22	3	**29.99**	**37.31**	**32.16**	**-**	**30.40**	**21.16**
23	2	**28.89**	40.00	**36.39**	**-**	**35.54**	**25.60**
24	5	**35.42**	**36.42**	40.00	**-**	**-**	**-**
25*	7	40.00	40.00	40.00	**-**	**-**	**-**
26	4	**34.60**	40.00	**39.67**	**-**	**27.93**	**22.46**
27	6	**29.54**	**35.05**	**26.97**	**-**	**37.20**	**21.50**
28	1	**35.90**	**37.55**	**37.08**	**-**	**33.03**	**23.75**
29	5	**32.74**	**35.93**	**35.58**	**-**	**-**	**-**
30	2	**34.80**	40.00	**30.70**	**-**	**31.68**	**22.02**
31	7	**34.00**	40.00	**35.55**	**-**	**30.52**	**20.93**
32	1	**31.58**	**37.44**	40.00	**-**	**-**	**-**
33	3	**27.04**	**36.09**	**24.02**	**-**	**-**	**-**
34	6	**32.22**	**35.21**	**35.31**	**-**	40.00	**30.69**
35	4	**31.71**	**37.41**	**34.21**	**-**	**-**	**-**
36	2	**34.66**	40.00	**38.78**	**-**	**-**	**-**
37	6	**31.81**	40.00	**36.88**	**-**	**-**	**-**
38	4	**31.38**	40.00	**31.45**	**-**	**35.58**	**22.06**
39	1	**30.64**	40.00	40.00	**-**	**31.29**	**24.28**
40	3	**34.25**	40.00	**33.54**	**-**	**33.83**	**21.99**
41	7	**32.01**	40.00	**36.99**	**-**	**-**	**-**
42	5	**35.93**	40.00	**35.93**	**-**	**-**	**-**
Positive control		30.75	29.15	18.93	30.75	29.15	18.93
Negative control		40.00	40.00	40.00	40.00	40.00	40.00
Mastermix		40.00	40.00	40.00	40.00	40.00	40.00

Bold values - Plots which have tested positive for root mat disease

- - Missing samples due to no symptomatic roots available to sample

* - Plots which tested negative for root mat disease

Mid-crop assessments

Trial A – The mid-crop assessment was performed on the 31st July 2017. Disease incidence had increased since the previous assessment with over 240 cubes symptomatic of the disease with a further 15 potentially infected cubes. Statistical analysis showed a similar pattern to the first symptom assessment. Vitix treated in propagation alone continued to show increased levels of root mat incidence with 13.3% of cubes affected per plot compared to just 3.8% in the untreated control (Figure 14, $P<0.001$). Interestingly, at this assessment, Vitix treated in crop alone also saw statistical increases in root mat disease incidence (9.8%). These results suggest that this effect was not an artefact of the low levels of disease seen at the earlier assessment and that Vitix is having a negative effect on root mat incidence regardless of when it was applied on this site. No differences were seen between plants

treated with Vitix in both propagation and crop, the Carbon Gold treatments, and the untreated control.

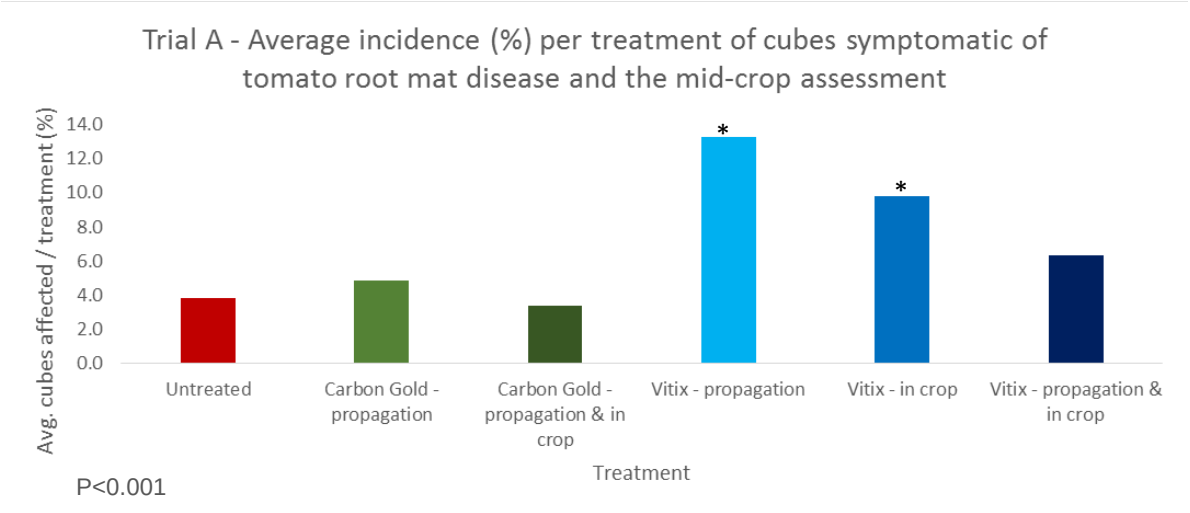


Figure 14. Incidence (%) of root mat disease in treated and untreated plots in Trial A at the mid-crop assessment – July 2017
*Statistically different to the untreated control

The distribution of severity scores at this assessment (Table 19) is similar to that of root mat disease incidence. Vitix continued to perform poorly, with the greatest severity scores in plots treated with Vitix in propagation alone, but also now in crop alone. Unlike the first assessment, all plots at this time showed visible symptoms, corresponding with the qPCR results taken at the first-symptom assessment.

Table 19. Distribution of severity scores (based on the percent of plants affected) of the five treatments and untreated control in Trial A at the mid-crop assessment – July 2017

Treatment	Severity score					
	1	2	3	4	5	6
Untreated	0.2	0.6	0.6	0.6	0.5	1.5
Carbon Gold - propagation	0.4	0.9	0.9	1.3	0.7	0.6
Carbon Gold - propagation & in crop	0.2	0.4	0.6	0.4	0.6	1.3
Vitix - propagation	0.2	2.8	2.6	1.5	2.4	3.7
Vitix - in crop	0.2	1.1	1.9	0.7	2.0	3.9
Vitix - propagation & in crop	0.2	1.3	1.1	0.9	0.9	1.9

Progression from green to red corresponds to larger numbers of cubes affected per treatment for each severity score

Analysis of the proportion of plots with a severity score corresponding to above 10% of cube surface spread, mirror the incidence results. This is unsurprising as Table 19 shows that at this assessment, a larger proportion of the recorded severity scores lie above three. No differences were seen between the untreated, either Carbon Gold treatments or Vitix treated in both propagation and crop combined. Vitix treated at propagation alone saw a three-fold increase in the proportion of cube coverage above 10% from 2.5% in the untreated to 7.6%, (Figure 15, P<0.001). Vitix treated in crop alone also increased the proportion, however to a lesser extent to 6.7%.

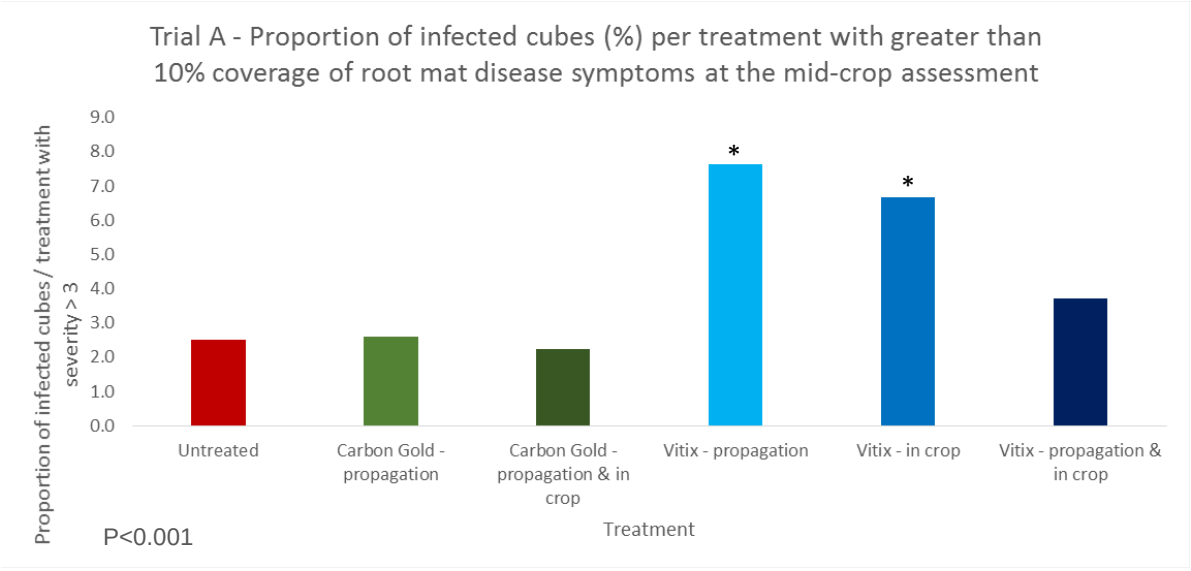


Figure 15 – The proportion of cubes from each treatment with root mat severity coverage greater than 10% in Trial A at the mid-crop assessment – July 2017

* Statistically different to the untreated control

Trials B and C - The mid-crop assessment of Trials B and C were performed on the 8 - 10th of September. This allowed a comparable time period between the first-symptom and mid-crop assessments at Trial A, due to the late arrival of the tomato plants on Site 2. The incidence of root mat disease in trial B (cv. Piccolo) in once-used slabs increased from 483 confirmed incidences to 1266, with 42% of total cubes in the trial now affected. Unlike at the first symptom assessment, statistical differences were seen at this date, as shown in Figure 16.

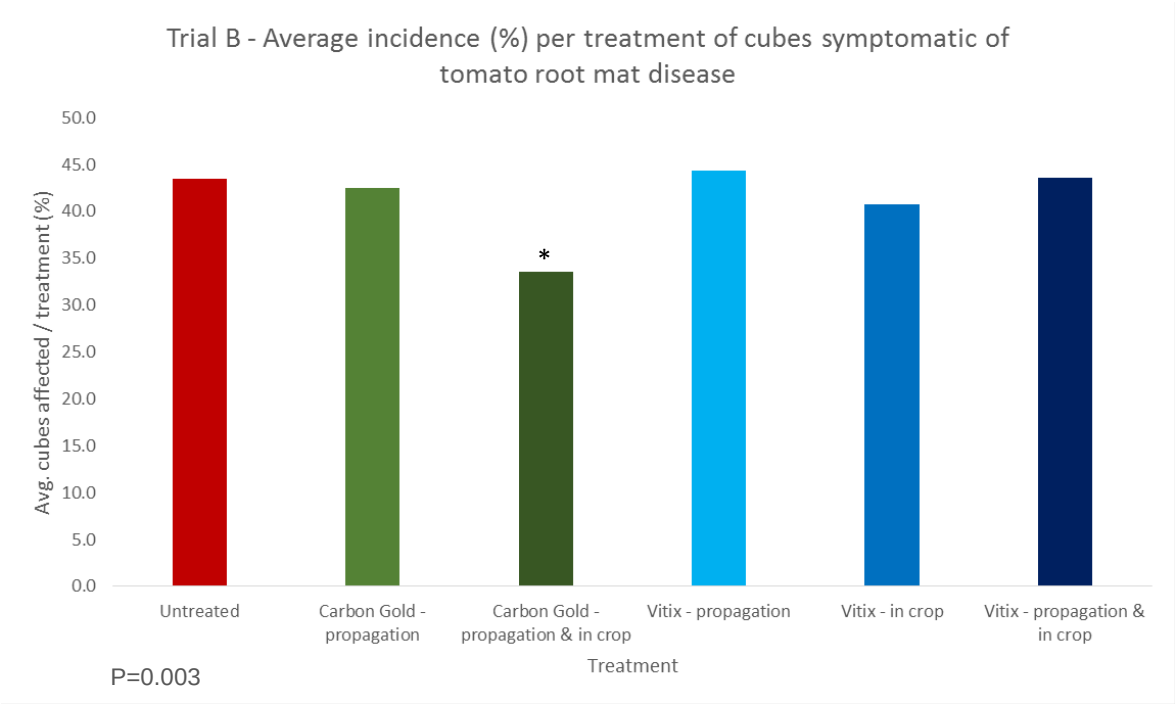


Figure 16 – Incidence (%) of root mat disease in treated and untreated plots in Trial B at the mid-crop assessment

*- Statistically different to the untreated control

Carbon Gold treated at both propagation and at planting reduced the incidence of tomato root mat disease in plants grown in second year bags by 10%, from 43.5% of cubes infected in the untreated to 33.5% (P=0.003). No other differences were seen in root mat incidence between treatments at this time. This is a promising result especially if the slabs did contain a viable *R. radiobacter* source at plant arrival. If this is the case, Carbon Gold treated at both propagation and in crop suppressed root mat disease incidence in what are effectively inoculated slabs.

Interestingly, the breakdown of severity scores per treatment in Table 20 reveals that the majority of infected cubes in each plot did not show severe symptoms, with most severity scores corresponding to below 10% of cube coverage.

Table 20 - Distribution of severity scores (based on the percent of plants affected) of the five treatments and untreated control in Trial B at the mid-crop assessment – September 2017

Treatment	Severity score					
	1	2	3	4	5	6
Untreated	10.5	14.0	9.5	7.7	1.6	0.2
Carbon Gold - propagation	15.6	11.0	9.8	5.6	0.4	0.0
Carbon Gold - propagation & in crop	16.5	6.0	6.9	3.3	0.8	0.0
Vitix - propagation	11.7	12.3	10.6	6.3	3.5	0.0
Vitix - in crop	10.8	13.3	10.8	5.5	0.5	0.0
Vitix - propagation & in crop	13.6	18.6	7.4	3.3	0.8	0.0

Progression from green to red corresponds to larger numbers of cubes affected per treatment for each severity score

Analysis of the proportion of severity scores greater than three shows that the untreated performed worst with 9.5% of cubes exceeding 10% coverage of root mat symptoms. All treatments in this trial, apart from Vitix treated in propagation alone, gave significant reductions in root mat severity scores above three at this mid-crop assessment (Figure 17, P<0.001). Interestingly, Vitix performed very similarly to Carbon Gold except when applied at propagation alone. Both Carbon Gold applied at propagation and Vitix applied in crop reduced the severity of cubes, with greater than 10% root mat coverage, from 9.5% in the untreated to 6%, a small but statistically significant reduction. Carbon Gold and Vitix when applied at both propagation and in crop both reduced severity to 4% at this date

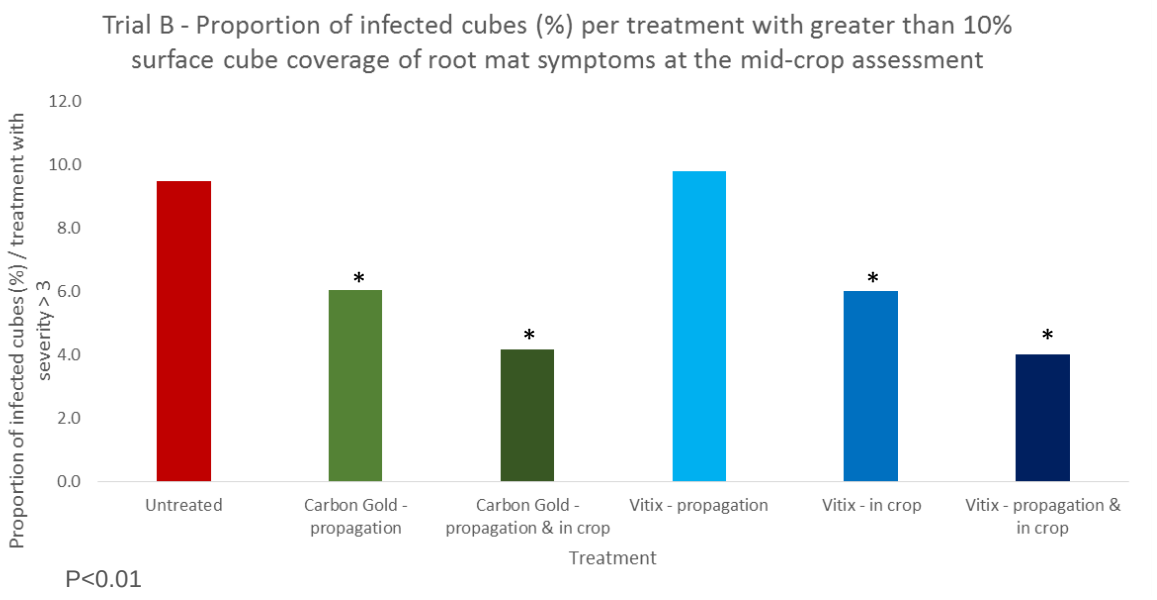


Figure 17 - The proportion of cubes from each treatment with root mat severity coverage greater than 10% in Trial B at the mid-crop assessment, no infection was detected in plants

when they arrived on the nursery in this trial. Rockwool cubes were planted onto once-used coir slabs with a history of the disease. – September 2017
*Statistically different to the untreated control

The incidence of confirmed root mat disease also increased at the mid-crop assessment in Trial C (cv. Funtelle on new slabs). In total, 426 cases of infected cubes were seen accounting for 15.1% of the total cubes in this trial. No treatments reduced the incidence of root mat disease compared to the untreated at this time (Figure 18, P=0.690).

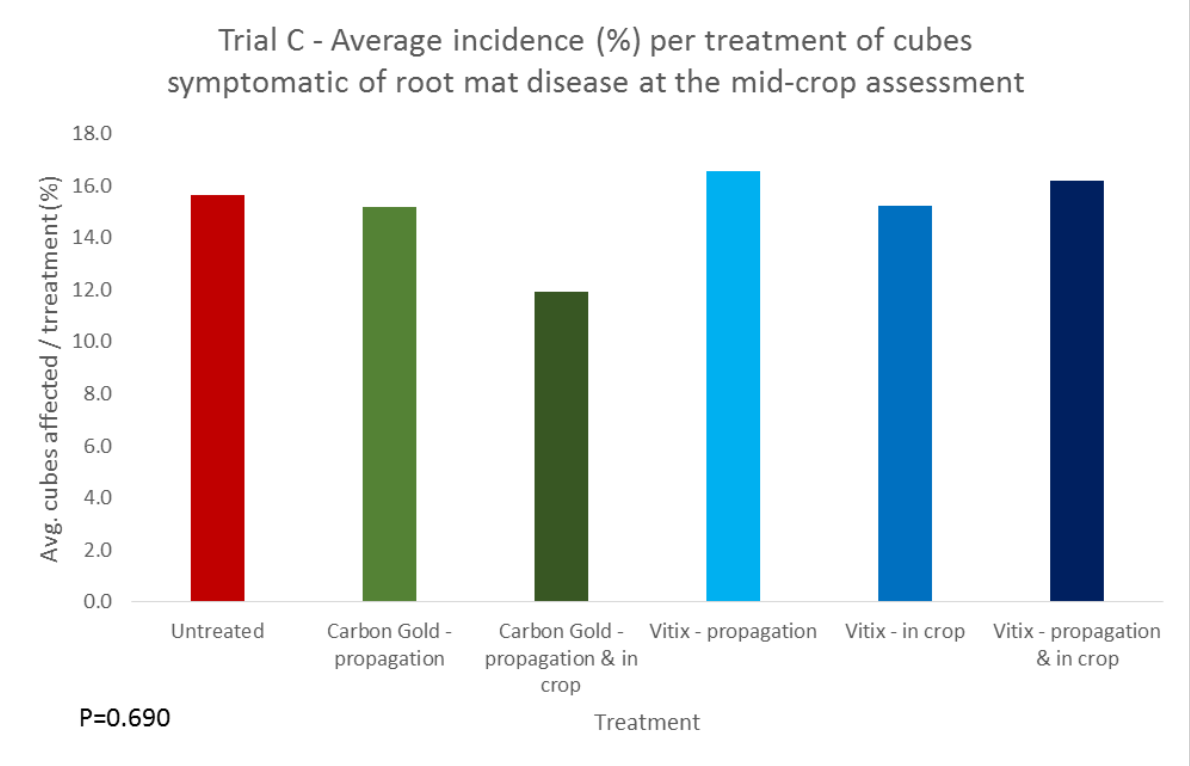


Figure 18 - Incidence (%) of root mat disease in treated and untreated plots in Trial C at the mid-crop assessment – September 2017

Interestingly, the distribution of severity scores differed in Trial C compared to Trial B with the most frequent scores being more severe despite the incidence of root mat disease being much lower. Very few low severity scores of 1, corresponding to 1-2 upright roots were recorded at this time (Table 21). It is possible that the slabs in Trial B had established microbial communities not found in new coir slabs. These could potentially contain antagonistic microorganisms which limit populations of *R. radiobacter* leading to the high levels of root mat disease incidence, but the relatively low severity scores recorded. Future work would be required to establish if this is the case.

Table 21 - Distribution of severity scores (based on the percent of plants affected) of the five treatments and untreated control in Trial C at the mid-crop assessment – September 2017

Treatment	Severity score					
	1	2	3	4	5	6
Untreated	0.2	1.6	3.5	3.9	3.9	2.7
Carbon Gold - propagation	0.0	0.9	3.8	5.4	2.0	3.1
Carbon Gold - propagation & in crop	0.6	1.5	2.6	3.7	3.0	0.5
Vitix - propagation	0.5	2.4	3.1	5.7	4.4	0.5
Vitix - in crop	0.6	2.0	3.7	3.7	3.1	2.2
Vitix - propagation & in crop	0.9	3.7	3.7	3.7	2.8	1.4

Progression from green to red corresponds to larger numbers of cubes affected per treatment for each severity score.

The incidence of root mat disease was much lower in Trial C than Trial B. The proportion of infected plots recording a severity score greater than three is larger than was seen in Trial B despite it being on the same site and in the neighbouring glasshouse (Figure 19). No treatments were effective in reducing the proportion of cubes with a surface coverage of greater than 10% root mat disease compared to the untreated at 10.4% (P=0.436).

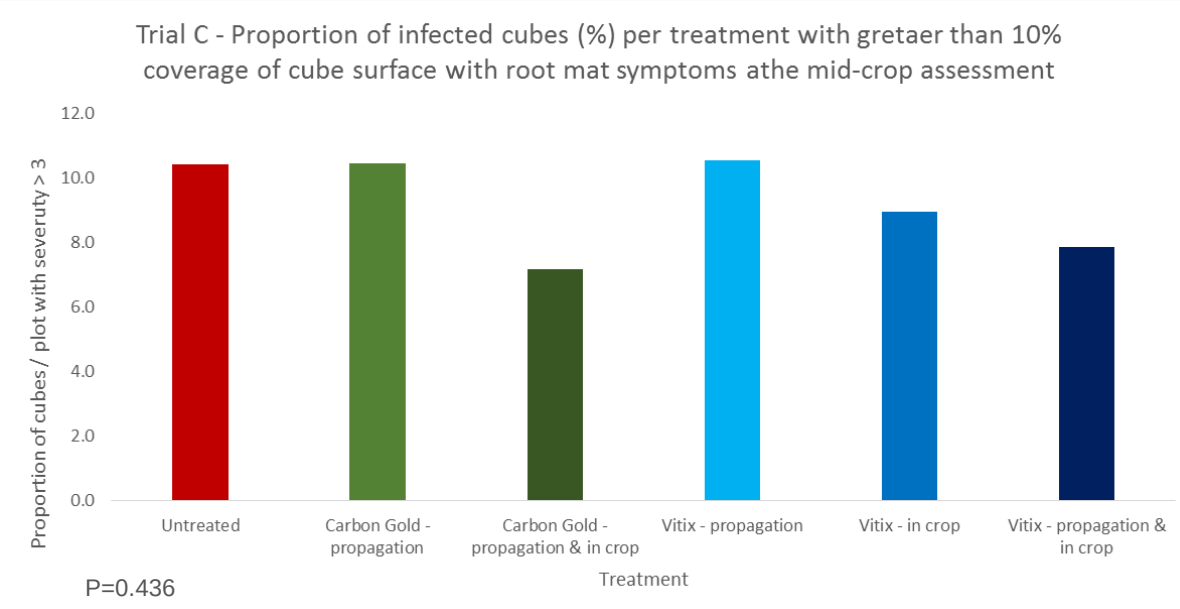


Figure 19 - The proportion of cubes from each treatment with root mat severity coverage greater than 10% in Trial C at the mid-crop assessment – September 2017

End-of-season assessments

The final assessment for Trial A was performed on the 9th of November 2017. Despite root mat disease being present within the crop for several months and this crop arriving in January

rather than March, the average disease incidence was lower at this site than both trials at Site 2, with only 4.9% of the untreated crop visibly symptomatic of root mat disease. Earlier qPCR results suggest that more plants may be infected but be asymptomatic of the disease.

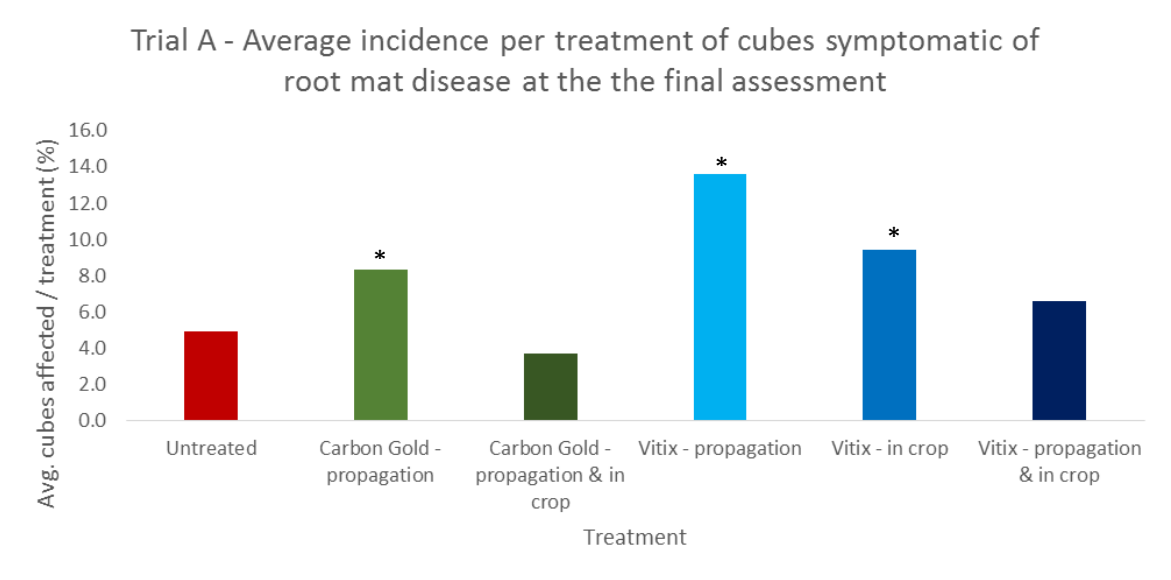


Figure 20 - Incidence (%) of root mat disease in treated and untreated plots in Trial A at the end of cropping assessment – November 2017
*Statistically significant increase compared to the untreated control

Vitix applied at propagation, as well as Vitix applied at cropping, continued to increase the incidence of root mat disease compared to the untreated control (Figure 20, $P<0.001$). At this assessment 13.6% of cubes treated with Vitix in propagation were affected with root mat disease, nearly three times that seen in the untreated control (4.9%). Vitix applied in crop alone also increased the incidence, but to the lesser extent of 9.4%.

For the first time in these trials, an increase in the incidence of root mat disease was also seen in plants treated with Carbon Gold at propagation alone. This was a small, but significant increase to 8.3% of cubes containing treated plants affected.

The severity distribution in Table 22 shows a continued shift towards more severe infections, with very few instances of low severity scores. The most common severity score for any treatment was Vitix treated at propagation alone, with 5.7% of treated plants showing 50% slab coverage compared to just 1.2% in the untreated.

Table 22 - Distribution of severity scores (based on the percent of plants affected) of the five treatments and untreated control in Trial A at the end-of-cropping assessment – November 2017

Treatment	Severity score					
	1	2	3	4	5	6
Untreated	0.2	0.3	0.7	1.2	1.3	1.2
Carbon Gold - propagation	0.4	0.7	3.3	0.9	1.5	1.5
Carbon Gold - propagation & in crop	0.0	0.2	1.1	0.4	0.7	1.3
Vitix - propagation	0.0	0.9	2.2	2.6	2.2	5.7
Vitix - in crop	0.0	0.4	2.0	0.4	3.3	3.3
Vitix - propagation & in crop	0.0	0.2	0.9	1.1	2.2	2.2

Progression from green to red corresponds to larger numbers of cubes affected per treatment for each severity score.

With few low severity scores, the proportion of cubes per treatment exhibiting greater than 10% of cube coverage with root mat symptoms is very similar to the incidence. Vitix treated in propagation increased the proportion scoring above three from 3.7% in the untreated to 10.5% (P<0.001, Figure 21). Vitix applied in crop which had a lesser effect on increasing disease incidence showed reduced severity compared to Vitix treated in propagation increasing the proportion to 7.0%.

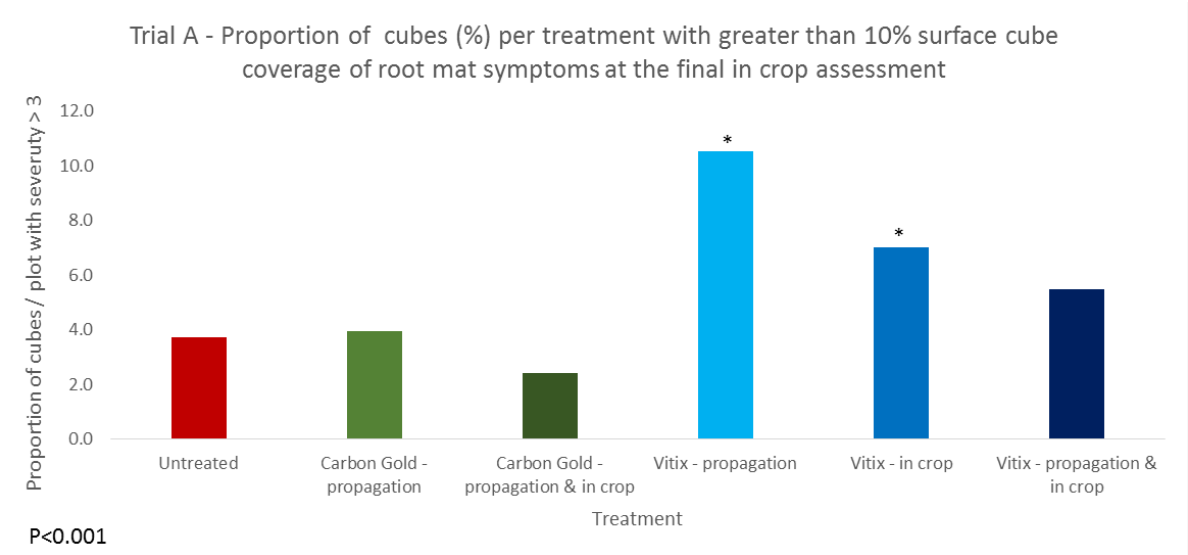


Figure 21 - The proportion of cubes from each treatment with root mat severity coverage greater than 10% in Trial A at the end-of-cropping assessment – November 2017
*- Statistically different to the untreated control

Whereas Carbon Gold treated at propagation gave a small increase in incidence this did not manifest as an increase in severity. No differences were found between Carbon Gold and the untreated at this or any severity assessment in Trial A.

The results from the first-symptom qPCR assay (May 2017) on symptomatic and asymptomatic roots revealed that all but two plots were infected with tomato root mat disease.

No disease was recorded in plot four at the end-of-cropping assessment, but disease was seen in two cubes in plot 15. Ten root samples from asymptomatic plants per plot were collected and analysed using the enrichment process by Fera Science Ltd. to determine whether plots contained infected but asymptomatic plants at this stage. The results (Table 23) confirmed that of the 10 asymptomatic samples collected from plots 4 and 15 the disease was not present. This result corresponds to that seen in the first set of in-crop qPCR results. Interestingly eight other plots recorded negative results for root mat disease in the bulked asymptomatic plant samples taken at this date, which was not seen in May. Experimental work indicates that younger plants are generally more susceptible to developing visual root mat disease symptoms than older more established plants (Bosmans L, *et al.,* 2017) and once a plant is infected it cannot be cured. This suggests that asymptomatic plants sampled at the first-symptom assessment were likely infected and had not yet developed symptoms and so tested positive. At the end-of-cropping assessment most plants which had infection would have developed symptoms and so only truly uninfected plants remained to be sampled resulting in the greater number of plots recording as negative for the disease. It is important to note that although most plants which were infected had likely developed root mat symptoms at this time, the majority of plots still had asymptomatic plants testing positive for the disease. This implies infection continues later on in the season, or infection had remained latent, with plants still not yet symptomatic. It also suggests the possibility that some plants may become infected but remain asymptomatic, which could be worth further investigation.

It is important to remember that only 10 asymptomatic root samples were taken from each plot at this time. Each plot in Trial A contained 160 cubes and it is possible that if a greater proportion of asymptomatic roots were sampled the disease may have been detected.

Table 23. qPCR results from asymptomatic bulked root samples taken at the end-of-cropping assessment, Trial A - November 2017

	Asymptomatic samples	
Plot	Pre-enrichment	Post-enrichment
1	40.00	**34.50**
2	40.00	**36.25**
3	40.00	**34.77**
*4	40.00	40.00
5	40.00	**30.09**
6	**33.45**	**21.92**
7	40.00	**25.76**
8	40.00	**34.57**
9	**35.63**	**38.05**

10	**36.07**	**24.52**
**11	40.00	40.00
12	40.00	**39.90**
13	40.00	40.00
14	40.00	**35.89**
*15	40.00	40.00
16	40.00	**29.53**
17	40.00	**33.53**
**18	40.00	40.00
19	40.00	**27.11**
20	40.00	**33.62**
**21	40.00	40.00
22	**33.48**	**28.61**
23	**35.02**	**31.81**
**24	40.00	40.00
25	**33.48**	**23.07**
26	**36.81**	**31.86**
**27	40.00	40.00
**28	40.00	40.00
29	40.00	**28.96**
30	**35.56**	**24.00**
31	40.00	**32.91**
32	40.00	**34.44**
33	40.00	**29.49**
34	**34.74**	**23.48**
35	40.00	**28.88**
36	**33.54**	**30.77**
37	40.00	**31.47**
38	40.00	**38.54**
39	**38.27**	**31.45**
40	40.00	**33.87**
**41	40.00	40.00
**42	40.00	40.00
Positive Control	**20.10**	**19.51**
Negative control	40.00	40.00
Mastermix	40.00	40.00

Bold values – plots which tested positive for root mat disease

* Asymptomatic root sample results from plots which tested negative for root mat disease at both the first-symptom and end-of-cropping assessment

** Asymptomatic root sample results from plots which tested negative for root mat disease at end-of-cropping, but positive at the first-symptom assessment

The final assessments for Trials B and C took place on the 14th - 16th of November at Site 2. In Trial B over half of all untreated plots were infected with root mat disease. Only one product reduced the incidence of root mat disease at this time, Carbon Gold treated at both propagation and at planting (Figure 22, P=0.002). This reduced the incidence by 10% from 54% in the untreated to 44%, a useful level of reduction. Carbon Gold treated at propagation and at planting performed similarly at the mid-crop assessment reducing the disease incidence by 10%, from 43% in the untreated to 33% in the once-used slabs. If a pre-existing inoculum source within the once-used slabs was responsible for the very high incidence results seen in this trial, it is advisable not to re-use previous infected slabs in future crops.

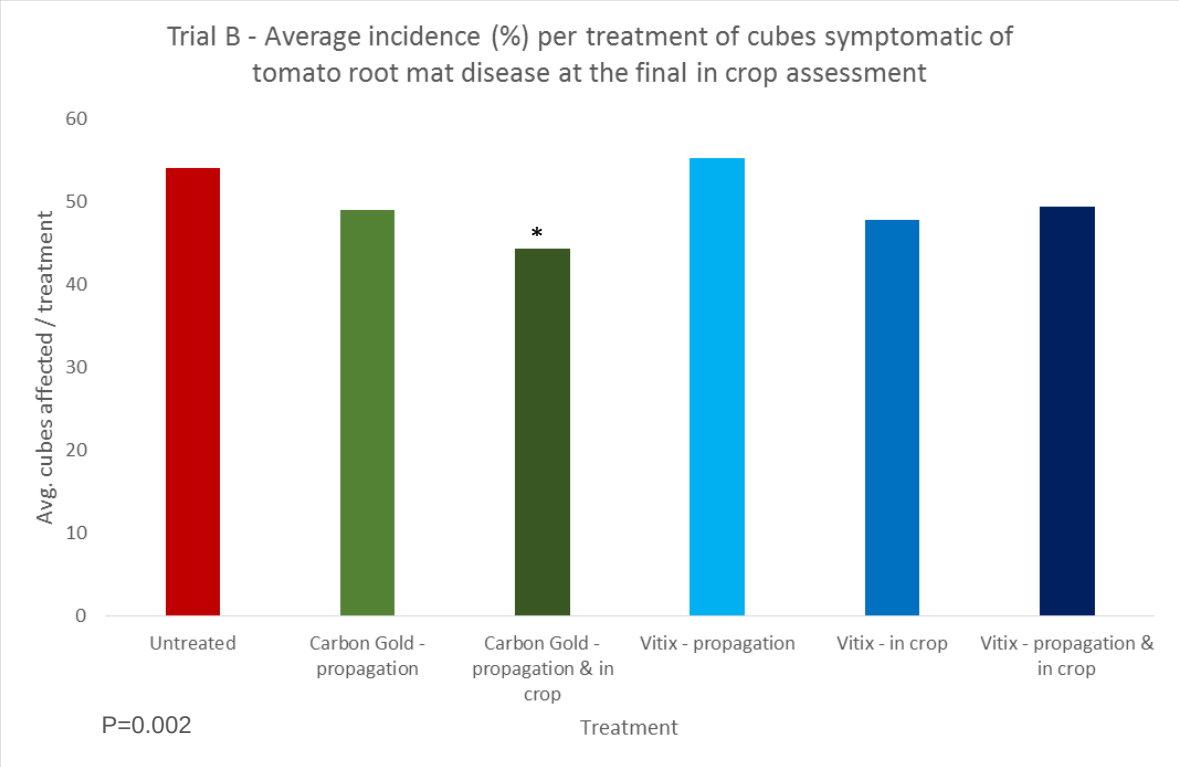


Figure 22 - Incidence (%) of root mat disease in treated and untreated plots in Trial B at the end-of-cropping assessment – November 2017
*Statistically different to the untreated control

Distribution of severity scores was uniform with fewer severity scores below three (Table 24). This is a similar situation to that seen in the mid-crop assessment, apart from the increased incidence of the disease. Carbon Gold treated at propagation and at planting showed the lowest severity scores recorded.

Table 24 - Distribution of severity scores (based on the percent of plants affected) of the five treatments and untreated control in Trial B at the end-of-cropping assessment – November 2017

Treatment	Severity score					
	1	2	3	4	5	6
Untreated	21.8	14.4	9.5	5.6	2.2	0.6
Carbon Gold - propagation	19.2	14.2	8.1	5.2	1.9	0.4
Carbon Gold - propagation & in crop	21.5	12.7	4.4	2.9	1.9	1.0
Vitix - propagation	13.3	21.0	10.0	7.1	3.1	0.6
Vitix - in crop	12.8	18.8	7.0	5.8	2.8	0.8
Vitix - propagation & in crop	22.4	12.9	7.9	4.7	1.3	0.3

Progression from green to red corresponds to larger numbers of cubes affected per treatment for each severity score.

With such low proportions of severity scores and the uniformity in these results it unsurprising that no significant differences in severity scores was seen between any treatments and the untreated control (Figure 23, P=0.05) The results were close to showing a reduction in severity score in plants treated with Carbon Gold at propagation and crop however. This was seen at the mid-crop assessment and it may be that a small significant reduction could have been seen if the assessment had been performed slightly earlier.

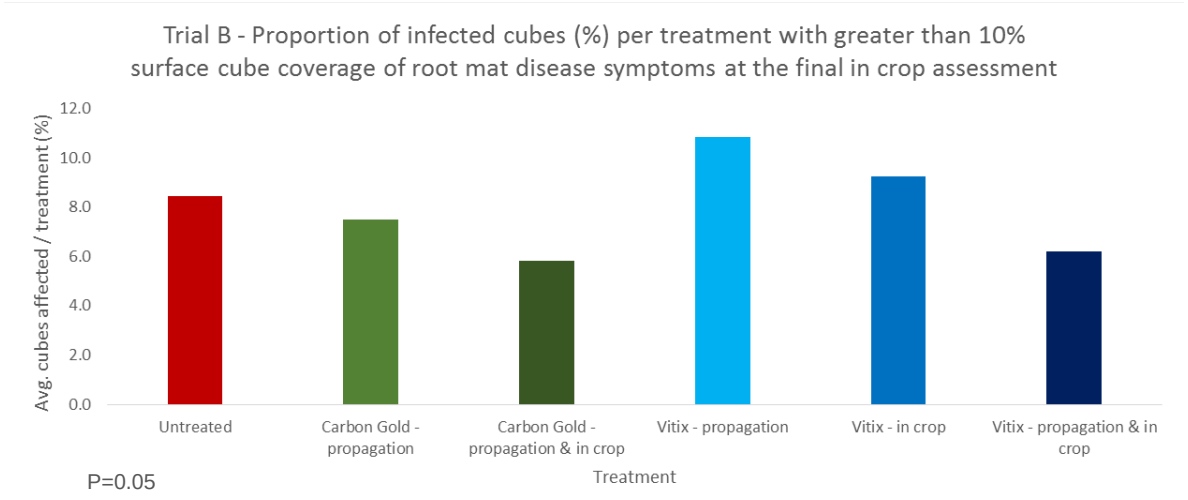


Figure 23 - The proportion of cubes from each treatment with root mat severity coverage greater than 10% in Trial B at the end-of-cropping assessment – November 2017

Trial C, planted on new slabs compared to Trial B on once-used slabs, showed a lower disease incidence at the final assessment, 34.8% compared to 54.1%. Only one treatment

showed a reduction in root mat incidence, Carbon Gold applied at propagation alone. This treatment reduced the root mat incidence from 34.8% in the untreated to just 16.8% in treated plants (Figure 24, P<0.001). This is a strong reduction in root mat incidence.

The incidence of root mat disease in plants treated with Carbon Gold at propagation at the mid-crop assessment in Trial C was 15.2% whilst the untreated was 15.7%. A large increase in disease incidence occurred between the mid-crop and final assessment, more than doubling incidence in the untreated to 34.8% in this time. What caused this is unknown, but root mat infection was confirmed in all but one plot in Trial C in June (Table 18) and environmental conditions may have enabled the disease to develop allowing previously asymptomatic plants to become symptomatic. Carbon Gold applied to plants at propagation allowed them to resist this, only suffering an increase in incidence from 15.2% to 16.8%.

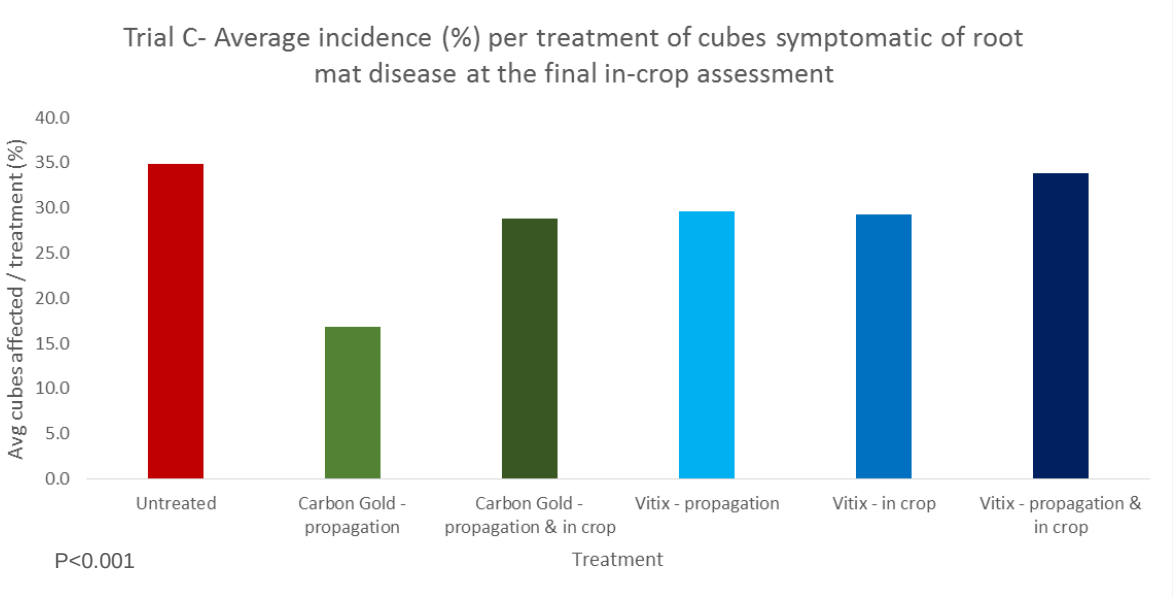


Figure 24 - Incidence (%) of root mat disease in treated and untreated plots in Trial C at the end-of-cropping assessment -14th-16th November 2017
*Statistically different to the untreated control

The score distribution in Table 25 shows that not only has the incidence of root mat disease increased but the proportion of each score has also shifted to greater severity scores compared to the mid-crop assessment. The majority of all the severity scores at this time scored three or above, with very fewer scoring one or two.

Table 25 - Distribution of severity scores (based on the percent of plants affected) of the five treatments and untreated control in Trial C at the end-of-cropping assessment – November 2017

Treatment	Severity score					
	1	2	3	4	5	6
Untreated	0.3	2.4	7.2	20.4	39.1	30.5
Carbon Gold - propagation	0.0	6.7	18.9	11.0	32.0	31.5
Carbon Gold - propagation & in crop	0.0	2.4	18.1	17.8	34.9	26.8
Vitix - propagation	0.0	4.7	6.3	36.4	33.9	18.7
Vitix - in crop	0.6	7.6	7.9	17.0	31.4	35.5
Vitix - propagation & in crop	2.1	1.1	6.9	19.5	39.7	30.7

Progression from green to red corresponds to larger numbers of cubes affected per treatment for each severity score.

At this end-of-cropping assessment, two products provided a reduction in root mat severity scores above 10% of cube surface coverage. Carbon Gold at propagation which significantly reduced incidence also reduced severity, decreasing the proportion of cubes affected above 10% from 30.6% in the untreated to 12.8%, a good level of reduction (Figure 25, P=0.001). Carbon Gold treated at propagation and crop also reduced the proportion of scores above three to a lesser, but still sizeable extent from 30.6% to 24.1%.

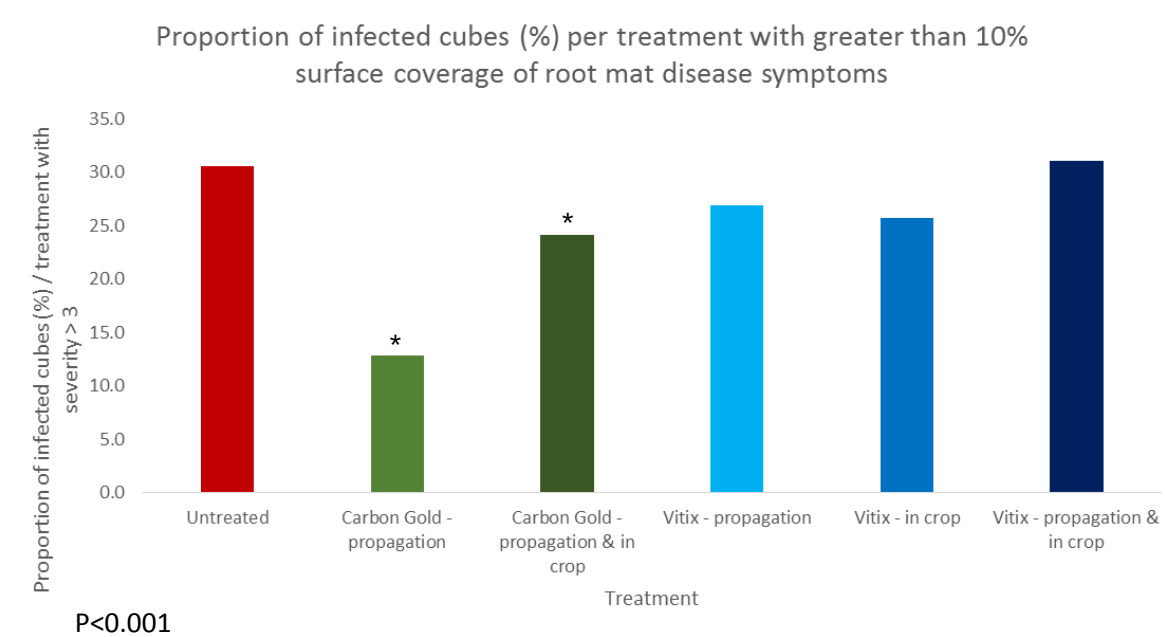


Figure 25 - The proportion of cubes from each treatment with root mat severity coverage greater than 10% in Trial C at the end-of-cropping assessment – November 2017
*Statistically different to the untreated control

Heatmaps of the distribution and spread of R. radiobacter during 2017

The heat maps for Trials A – C are located in Appendix 3 and show the distribution and spread of tomato root mat disease over the course of the season. The grey cells represent rows / proportion of rows not assessed due to treatment issues at propagation.

Trial A (Site 1) had the lowest incidence of any trials at both assessment dates. The initial spread of the disease looks random, however distinct clusters of the disease can be seen by the end-of-cropping assessment. A clear line is visible in plot 24 in plants treated with Vitix at propagation alone. By the end of the season the majority of the infected cubes showed severe infection (dark orange to red) with very few showing low severity scores (yellow).

The heatmaps for Trial B (Site 2) reveals that much greater levels of disease was recorded than in Trial A, even at the final assessment. The trial set-up is split over the central walkway and shows a distinct difference in the quantity of diseased plants in each half. The right-hand side (RHS), corresponding to the bottom half of the heat map has very few incidences of root mat disease with the left hand side (LHS) having the majority. By the end-of-cropping assessments the differences between the two halves of the trial were similar. No patterns in infection are visible at the final assessment. If the disease originated from a pre-existing inoculum in the once-used slabs this may go some way to explain this lack of difference by November. Despite much greater levels of infection the severity scores were much lower than those seen in Trials A and C at this time.

Trial C, also situated at Site 2, showed less infection than Trial B, but much more than in Trial A by the end-of-cropping. The same distribution, with the majority of infection occurring on the LHS compared to the RHS is visible. Unlike in Trial B this pattern continued to the final assessment suggesting whatever caused this difference persisted. At this assessment, almost all infected plots showed quite significant levels of infection, with very few new incidences recorded.

The grower at Site 2 was questioned regarding the split distribution of the disease over the central walkway. Both the RHS in Trial B and the neighbouring Trial C is warmer than the LHS where much more disease was observed. Only one environmental data logger was placed within Trials B and C and so the differences in environmental conditions cannot be determined. It is therefore possible that temperature may have a role in the differences seen and may warrant further investigation in the future.

<u>Objective 5 - To determine the efficacy of some biocides used at crop turnaround in reduction of Rhizobium populations and Ri plasmid</u>

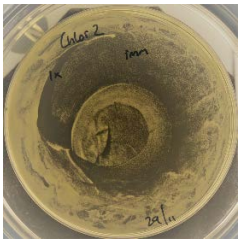
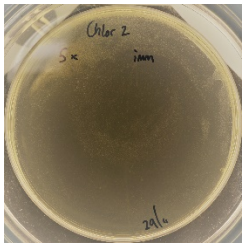
Experiment 1 – Biocide efficacy testing on pure R. radiobacter cultures

At the doses studied, the most effective biocide was Domestos Extended Power, achieving 100% kill of the bacterium at all concentrations used (Tables 26 and Figure 26). Clorious$_2$ was also 100% effective at the recommended dose and at 0.5x the recommended dose, but only if the exposure time was 2 min or more. No effect of Clorious$_2$ was observed at 0.1x the recommended dose, whereas, the minimum effective dose of Domestos was not determined in this experiment. It is important to note that the recommended dose of Clorious$_2$ (0.75 ppm 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. The hydrogen peroxide/stabilised silver biocides Geosil and Endosan 3 had no effect on the target bacterium at their recommended rates of 50ppm H_2O_2, or at 2x these rates, when compared with the water control. At 10x the recommended rates, some effect was observed which increased with exposure times above 5 min.

Table 26. The effect of exposure time and concentration of Clorious$_2$, Domestos Extended Power, Geosil and Endosan3 on the regrowth of pure cultures of *R. radiobacter*, December 2017

Clorious$_2$															
Exposure (min)	10X (7.5 ppm ClO_2)			5X (3.75 ppm ClO_2)			1X (0.75 ppm ClO_2)			0.5X (0.375 ppm ClO_2)			0.1X (0.075 ppm)		
0	-	-	-	-	-	-	+	+	+	++	++	++	+++	+++	+++
2	-	-	-	-	-	-	-	-	-	-	-	-	+++	+++	+++
5	-	-	-	-	-	-	-	-	-	-	-	-	+++	+++	+++
10	-	-	-	-	-	-	-	-	-	-	-	-	+++	+++	+++
Domestos Extended Power															
Exposure (min)	10X (16200 ppm NaClO)			5X (810 ppm NaClO)			1X (1620 ppm NaClO)			0.5X (810 ppm NaClO)			0.1X (162 ppm NaClO)		
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Geosil															
Exposure (min)	10X (500 ppm H_2O_2)			5X (250 ppm H_2O_2)			1X (50 ppm H_2O_2)			0.5X (25 ppm H_2O_2)			0.1X (5 ppm H_2O_2)		
0	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
5	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
10	+	+	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Endosan3															
Exposure (min)	10X (500 ppm H_2O_2)			5X (250 ppm H_2O_2)			1X (50 ppm H_2O_2)			0.5X (25 ppm H_2O_2)			0.1X (5 ppm H_2O_2)		
0	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
5	+	+	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
10	(+)	(+)	(+)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Water control															
Exposure (min)															
0	+++	+++	+++												
2	+++	+++	+++												
5	+++	+++	+++												
10	+++	+++	+++												

- No regrowth of *R. radiobacter* after treatment
+++ Full regrowth of *R. radiobacter* (10^7 CFU per ml) after treatment equivalent to water treated control
++ Reduced regrowth of *R. radiobacter* (10^4-10^6 CFU per ml) after treatment compared with water treated control
+ Reduced regrowth of *R. radiobacter* (10^2-10^4 CFU per ml) after treatment compared with water treated control
(+) Reduced regrowth of *R. radiobacter* (10-10^2 CFU per ml) after treatment compared with water treated control

Clorious$_2$	0.075 ppm ClO_2 - 0 min 	0.375 ppm ClO_2 - 0 min 		

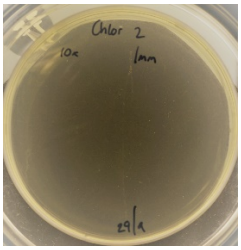
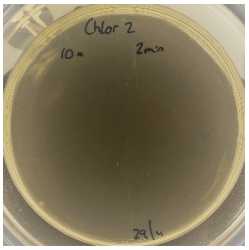
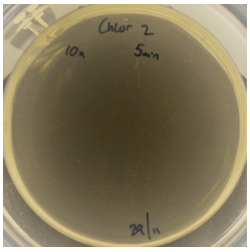
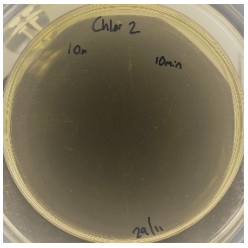
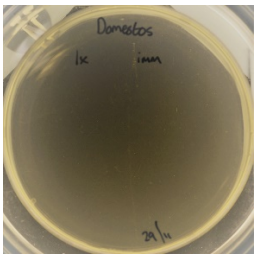
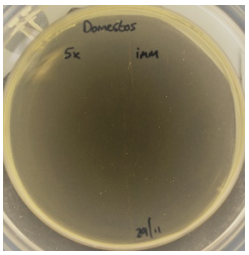
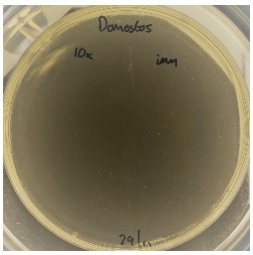
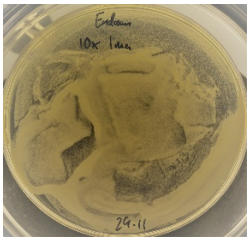
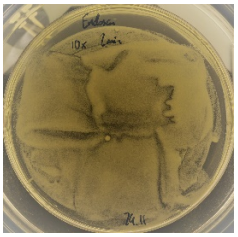
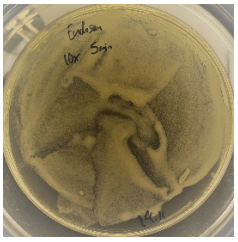
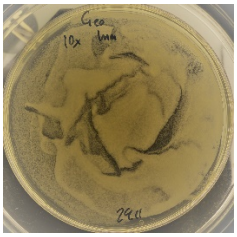
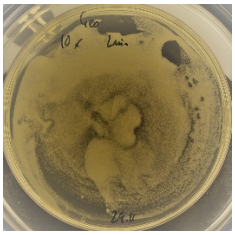
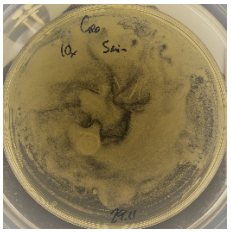
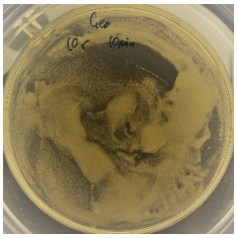
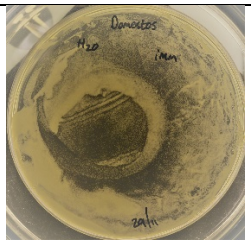
Clorious$_2$	0.75 ppm ClO_2 - 0 min 	0.75 ppm ClO_2 - 2 min 	0.75 ppm ClO_2 - 5 min 	0.75 ppm ClO_2 - 10 min 
Domestos	162 ppm NaClO - 0 min 	810 ppm NaClO - 0 min 	1620 ppm NaClO - 0 min 	
Endosan3	50 ppm H_2O_2- 0 min 	50 ppm H_2O_2- 2 min 	50 ppm H_2O_2- 5 min 	50 ppm H_2O_2- 10 min 
Geosil	50 ppm H_2O_2- 0 min 	50 ppm H_2O_2- 2 min 	50 ppm H_2O_2- 5 min 	50 ppm H_2O_2- 10 min 
H_2O				

Figure 26. Regrowth of *Rhizobium radiobacter* on nutrient dextrose agar following different biocide treatments

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

radiobacter

Of the four biocides tested, only Domestos Extended Power was effective at preventing further multiplication of *R. radiobacter* at the concentrations and exposure times used (Figures 27, 28 and 29). 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. This indicates that some of the bacteria in the biofilms remained protected from activity of the biocides rather than that there was 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). Further investigation will be needed to determine whether the other biocides can successfully remove viable biofilm by increasing doses and/or exposure times.

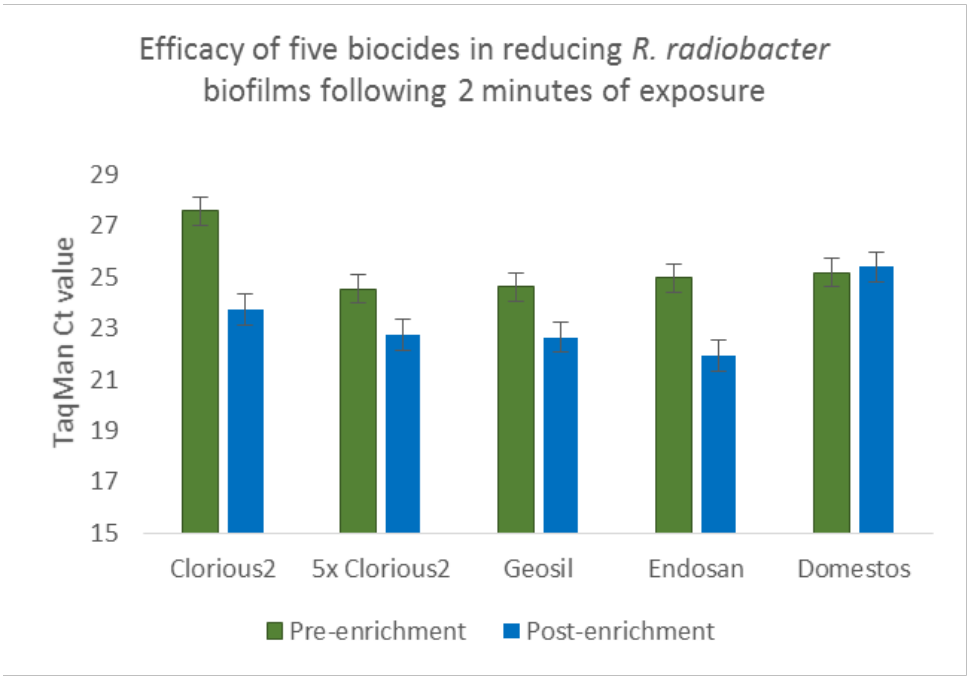


Figure 27. TaqMan qPCR results before and after enrichment of irrigation piping smeared with *R. radiobacter* following disinfection with different five biocides following a 2 minute exposure period, December 2017

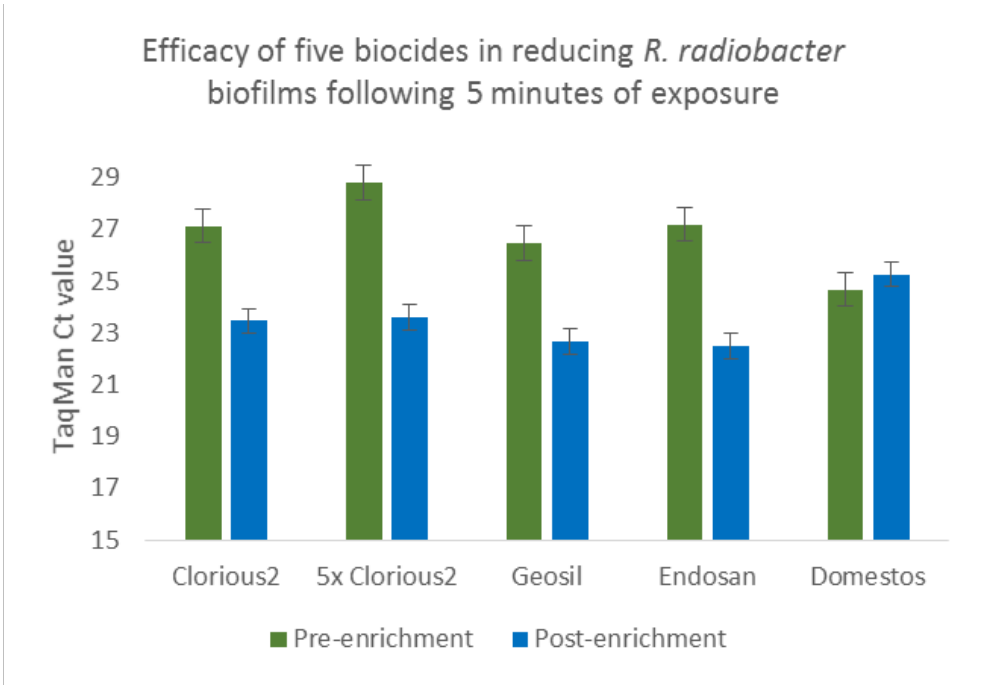


Figure 28. TaqMan qPCR results before and after enrichment of irrigation piping smeared with *R. radiobacter* following disinfection with different five biocides following a 5 minute exposure period, December 2017

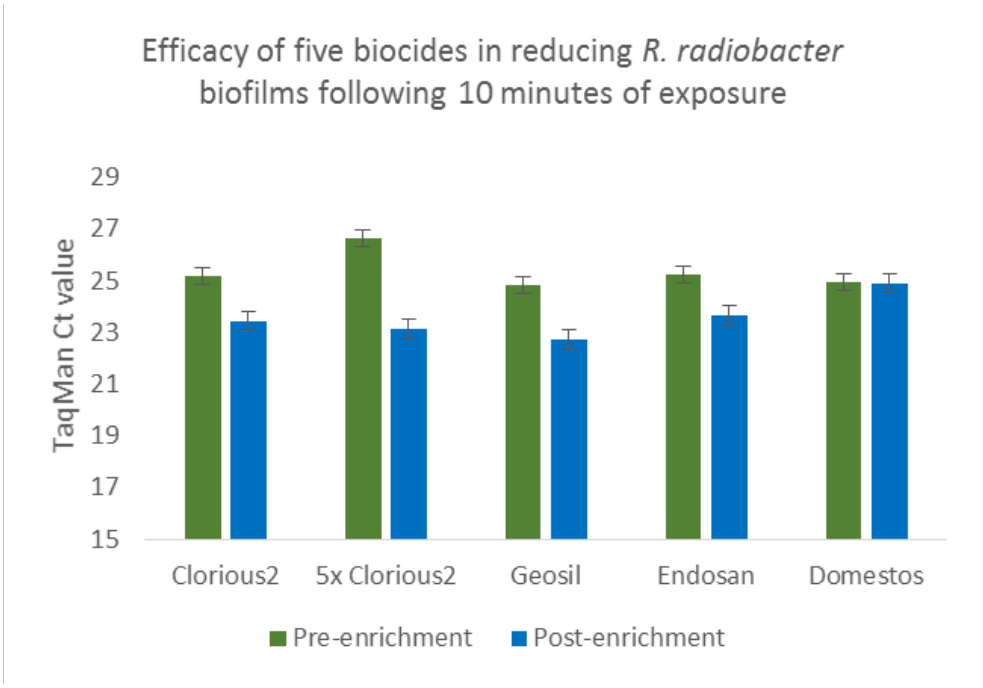


Figure 29. TaqMan qPCR results before and after enrichment of irrigation piping smeared with *R. radiobacter* following disinfection with different five biocides following a 10 minute exposure period, December 2017

Additional observations

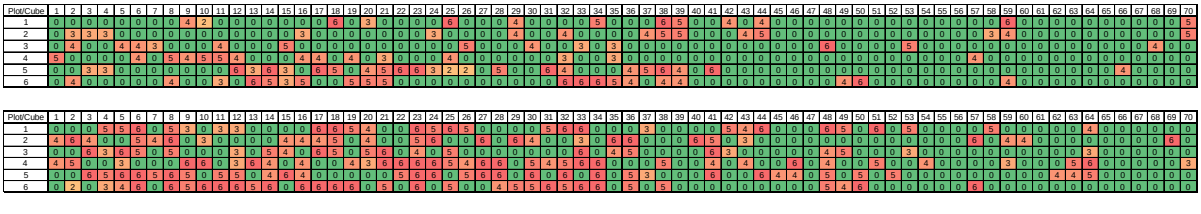
1. *Potential impacts of irrigation systems on the spread and control of tomato root mat disease*

An additional area of crop was treated with a greater rate of Vitix than that used in the neighbouring Trial C by the host grower at Site 2. This was observed to see if the greater rate of Vitix application had a positive effect on root mat incidence. It is important to note that observations are anecdotal as this is not a scientifically robust trial, rather a block of a single treatment which might offer insights complementary to the main trial work.

Figures 30 and 31 produced at the same dates as the mid-crop (July) and end-of-cropping (November) assessments revealed an unusual distribution of the spread of root mat disease incidence. Splitting the rows in half down the centre (35 plots each side) revealed that the incidence of the disease on the left-hand side (LHS) was roughly half of what it is on the right-hand side (RHS) at both assessment dates. This suggests something is having an effect on root mat disease incidence in this block.

All the plants in this block are fed from the same irrigation valve, and all receive the same water. The set-up of the irrigation system is such that each row is irrigated from both the back and the front of each row simultaneously meeting in the centre. A result of this is that the water destined for the back half of the rows has a much greater distance to travel before it leaves the system. When this system is considered with the unusual distribution of disease incidence it suggests that the irrigation system and/or quality of the water may have a role to play in spread, and subsequent control, of root mat disease. A diagram of block and the irrigation system is located in Appendix 4 with comments in Appendix 6.

Figures 30 and 31. The distribution and severity scores of tomato root mat disease in an additionally treated block of Vitix next to Trial C at the mid-crop (August 2017) and end-of-cropping (November 2017) assessments



Green cells correspond to uninfected cubes. Yellow though to red relate to infected cubes of increasing severity, yellow = low (1-2 raised roots on the cube surface, red = high (50% of cube surface affected

2. *Brown root rot assessment*

Severe infections of root mat disease can increase the likelihood of developing secondary root rots, such as those caused by pythium, phytopthora and *Pseudomonas* species (Weller *et al.,* 2006)). Dead / dying brown roots are characteristic of these secondary infections, due to necrotic root tissue. Brown roots were observed in Trial A, but this was the result of iron precipitation from the irrigation system rather than disease (Figure 32). Brown roots were observed in Trial B (Figure 33), but not Trial C.

Figure 32 – Brown roots in Trial A, a result of iron precipitation from irrigation water rather than root tissue infection / necrosis.

Figure 33 – Brown roots in Trial B, dead / dying root tissue from new or second year roots

The presence of brown roots in Trial B is recorded in Table 20. Statistical analysis did not identify any differences between the proportions of cubes containing brown roots in any treatment compared to the untreated (Table 27, P=0.306). During 2017, the disease never reached sufficient levels in Trials A and C for secondary root rots to become an issue, and no incidences were seen. The brown roots in Trial B are most likely the result of old dead / dying tissue remaining from year one in the second year slabs which would also explain why no treatment had an effect. No symptoms of secondary rots were seen in Trial B during the 2017 season. Occurrence of dead plants at the final assessment was very low (<1%) with no differences seen between treatments.

Table 27 - The % of cubes per treatment in Trial B which showed brown roots – July 2017

Treatment	% plots with brown roots
Untreated (average)	15.6
Carbon Gold - propagation	12.5

Carbon Gold - in propagation & crop	12.3
Vitix - propagation	19.8
Vitix - in crop	11.8
Vitix - propagation & in crop	13.6

Discussion

The effect of biological products applied at propagation, planting and during cropping on the incidence and severity of tomato root mat disease

Seed and water samples sent from the two Dutch propagators providing plant material for the three trials all tested negative for both *R. radiobacter* and the Ri plasmid. However, root samples taken for qPCR analysis at plant arrival on the commercial sites revealed that several treatments were infected with the root mat pathogen at this time although not symptomatic. It is not possible to determine when infection occurred, whether at propagation or during transport, but it did occur before or immediately upon arrival on site. It is also possible that in the samples which tested negative at propagation, infection could have been present, but at incidences too low to be detected by sampling for the qPCR assay used. If a proportion of plants are already infected by pRi on arrival on a production nursery, albeit latently, this suggests that root mat can be reintroduced to sites each year. Comprehensive site clean-up at turn around and good hygiene practices are crucial in effective disease management and disease reintroduction would undermine this, especially at this early stage. Further testing is required to determine how frequently batches of young plants are already infected by pRi at arrival on a nursery.

Root samples collected from plants in Trial B all tested negative for infection at plant arrival whilst both the Carbon Gold and Vitix treated plants used in Trial C tested positive for the disease. This is an interesting result as the plants for both Trials B and C were sourced from the same Dutch propagator. The Dutch propagators used by both sites were contacted and confirmed that each block of treated plants were kept separate at the propagation stage. If infection did occur at propagation then this might explain the differences in infections observed. If infection did not occur at propagation it may have occurred during distribution to the commercial sites and this process should be investigated e.g. swab testing of delivery vehicle, plant trays etc. for *R. radiobacter* and pRi presence.

The qPCR testing of symptomatic and asymptomatic roots at the first symptom assessment revealed that despite very low disease incidence in Trials A and C, almost every plot was infected with the root mat pathogen. Every plot in Trial B was confirmed to be infected at this stage regardless of whether asymptomatic or symptomatic roots were tested. In Trial A, plants treated with Vitix at propagation alone, and in crop alone, showed an increase in disease incidence compared to the untreated control. This suggests the bacterium is present on a large proportion of plants in all treatments without symptoms developing and that the Vitix treatment allowed this to occur or increased the speed of occurrence. It is plausible that by the end of the growing season every plant is infected with root mat disease to some extent, with only a proportion becoming symptomatic and this is of interest for future investigation. This could be due to varying population levels of *R. radiobacter* containing the Ri plasmid not reaching sufficient levels for a quorum, the population of *R. radiobacter* required for activation of pathogenicity; and / or environmental factors, including plant growth stage, not being favourable for symptom expression. Cubes suffering over 50% of root mat coverage are sometimes found next to a cube asymptomatic of the disease and occasionally the slab will show infection with no disease present on either cube, normally radiating from a drip peg in the centre of the slab. Identifying why some infected plants exhibit severe infections whilst others, which could still be infected, do not, may provide insights into root mat disease control. In Trials A and C at the end-of-cropping assessments in November, few low severity scores of 1 and 2 were recorded. This suggests that low instances of new infections were occurring at this time and that pre-existing infections were becoming more severe. Older plants are less susceptible to the disease than young plants and this could be a reflection of this.

Trial B was placed on once-used coir slabs with a history of the disease at Site 2. The root samples collected from both treatments and the untreated control tested negative for root mat disease at plant arrival. The grower at this site has in the past successfully used the same coir slabs for multiple years claiming better yields as a result. It is worth noting that Site 2 had never used once-used slabs with a history of root mat disease before. It is entirely possible that viable *R. radiobacter* was still present in these slabs to some degree from the previous year allowing rapid infection of this clean Piccolo crop, effectively making this an ‘inoculated’ commercial trial. This would explain the large incidence of root mat disease seen in Trial B compared to Trials A and C. Despite a much larger incidence of root mat disease, the proportion of severity scores above 10% at the final assessment (as a proportion of infected cubes) was found to be much lower than in Trials A and C. Complex microbial communities have been shown in many environments to have a suppressive role, limiting pathogen populations through occupation of all available ecological niches (Beredsen R., *et al.,* 2012)

As the once-used slabs were not sterilised at Site 2, it is possible that communities could have formed which have this effect on *R. radiobacter* populations. This may explain the high levels of incidence observed, but also the lower proportion of severity scores above 10% cube coverage recorded.

In two of the three commercial glasshouse trials in 2017, Carbon Gold was shown to reduce the incidence and severity of tomato root mat disease. Carbon Gold Biology Blend is a highly porous compound which provides a habitat for microorganisms, including mycorrhizal fungi and *Trichoderma* sp. These micro-organisms may be antagonistic to pathogenic bacteria / fungi such as *R. radiobacter* through either competition for available ecological niches or directly killing the bacterium. Although Carbon Gold provided a benefit in reducing root mat disease to a commercially useful extent in two of the three trials, results were inconclusive as to which treatment combination / timing was best. Trial C containing cv. Funtelle saw a large reduction in disease incidence compared to the untreated control (by 50%) in plants treated at propagation alone, but this was not seen in plants treated with Carbon Gold at both propagation and in-crop. Trial B showed the opposite with Carbon Gold reducing the incidence when treated at both propagation and crop, rather than at just at propagation alone. It is unknown what is responsible for this effect, further work planned in 2018 on two trials (cv. Piccolo) grown on rootstock on new, unused slabs will further examine the benefits of different application timings of Carbon Gold.

At the mid-crop assessment in Trial B, plants treated with Vitix did see a reduction in severity scores, but not in disease incidence. This was the only positive result for Vitix over the course of the season in all three trials. The reduction in severity was small but significant, performing similar to that of Carbon Gold. Neither Carbon Gold nor Vitix had any effect on the severity of root mat disease at the final assessment. Carbon Gold did however reduce the incidence of root mat disease at the mid-crop and end-of-season assessments. A reduction in severity scores without a corresponding reduction in disease incidence is still a positive result. Plants with less severe symptoms can still be steered and uniformity maintained.

Vitix is a microbial biostimulant containing plant growth promoting microorganisms (mycorrhiza sp., *Trichoderma* spp., *Bacillus* spp.). It is reported to trigger rooting and enhance plant growth from the early stages of the crop. In the 2017 trials, Vitix did not reduce root mat disease incidence in any of the three trials, and in Trial A Vitix treated in propagation alone, and in crop alone, actually increased root mat disease incidence and subsequently, the severity. The qPCR testing of root material at plant arrival in Trial A showed no infection in plants treated with Vitix. This suggests that plants became infected on site from a pre-existing

inoculum source. *R. radiobacter* has been shown to migrate towards phenols released by wounding on plant roots. Once attached, the bacterium transfers part of the Ri plasmid T-DNA to the host root cell leading to cell transformation and root mat symptom development. Earlier work in this project during 2016 revealed that rough handling of the cubes was sufficient to lead to infection in inoculated plants. It is possible that the induction of rooting by the Vitix treatment could create small 'micro wounds' which are sufficient to attract the bacterium to infect. Unlike Carbon Gold, Vitix has to be applied repeatedly throughout the whole season. Multiple applications may lead to several 'micro wounding' events resulting in the high incidence observed in Trial A. This would also explain the fact that greater levels of infection compared to the untreated also occurred in plants treated with Vitix in crop alone.

It is possible that the Vitix microbial load applied at propagation was too great and this may have had a negative impact on either the health of the plant or the root zone environment itself. The quantity of Vitix applied may have led to a reduction in other beneficial species or acted as a burden competing with the plant roots for nutrients. Bacterial populations tend to increase more rapidly than other microorganisms and *R. radiobacter* could potentially have become established quickly with little or no competition at an early stage.

Wetter slabs are generally associated with increased risk of root mat disease development. It has been suggested that it is not the wetness of the slabs but the irrigation regime, which has an impact on root mat development. Each irrigation timing may trigger 'vegetative events' leading to root division and potentially infection. The UK uses some of the lowest slab volumes in Europe and as a result of this require more frequent irrigation inputs. Larger slabs hold more water and would reduce the number of irrigation events required, reducing these 'vegetative events' allowing the crop to remain generative reducing the risk of infection. An examination of different slab sizes and irrigation regimes on disease incidence and severity at host sites is required to establish this effect.

Additional work in this project indicates that the irrigation systems on commercial nurseries may play a role in the spread and control of root mat disease. The distribution of the incidence of root mat disease in an area of cv. Funtelle treated with additional Vitix showed a reduction in one half of the rows compared to another where the rows were irrigated from both ends. No statistical analysis could be performed as this was a block of one treatment rather than a randomised trial design.

The distribution in incidence observed could be an effect of the efficiency of disinfection of the irrigation system prior to planting, i.e. residual biofilm surviving in certain areas of the irrigation system e.g. at the ends. Many biocides used at crop turnaround are hydrogen

peroxide (H_2O_2) based and H_2O_2 concentrations in the irrigation circuit decrease with distance as it interacts with biofilms in the piping. Lower H_2O_2 concentrations measured at the end of the circuit correspond with a higher disease incidence at the end (Bosmans L. *et al.,* 2017).

It has also been proposed that levels of dissolved oxygen may influence root mat disease infection and/or development, with greater levels supressing bacterial biofilm growth which may be the significant source of *R. radiobacter* inoculum between seasons on sites. Although predominantly aerobic in nature some strains are capable of anaerobic respiration in the presence of nitrate and several strains are capable of growing at reduced oxygen levels (J Elphinstone, pers. Comm.).

More vegetative tomato varieties typically suffer more severe infections of tomato root mat disease compared to generative varieties. It is theorised that this is due to increased root division and possibly corresponding root damage which attracts *R. radiobacter* to infect. Steering a badly infected vegetative crop to become more generative has been reported to improve crop management at one nursery in Europe (Andy Lee, pers. comm). Plants used in Trials B and C arrived later than is standard for commercial sites. As a result of this, the grower was forced to steer these crops to be generative from the start which may have had a positive impact on reducing root mat disease at Site 2. Plants at this site were not grown on rootstock which may have also reduced the chances of infection due to less vigorous growth a reduction in tissue wounding.

Site 2 suffered severe infections of root mat in several other varieties grown in once-used slabs with a history of root mat disease infection. This led to the development of severe surface symptoms on both the propagation cubes and slabs leading to difficulty with crop steering, uniformity and an increase in out of specification fruit and the proportion suffering blossom end rot. The host grower applied routine calcium sprays and added supplementary coir on top of the slabs to aid in water uptake. Additional irrigation pegs were also put in the centre of the slabs to aid with irrigation. Although these measures did not restore the crop to an optimal condition they did pull it back to a more manageable situation. This indicates that it is possible to manage a heavily infected crop back ‘to relative health’ to some degree through cultural practices.

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

The two laboratory-based experiments to determine the efficacy of four biocides against *R. radiobacter* revealed some interesting results. The chlorine dioxide-based product Clorious$_2$ outperformed the hydrogen peroxide-based products in the ability to kill pure cultures of the

bacterium at every rate and exposure length. Chlorination has long been used as a method of disinfection of water by preventing bacterial growth. This work has shown that chlorine containing products such as Clorious$_2$ represent the best available biocides, of those tested in this study, to treat against root mat disease. When applied at full-rate, Clorious$_2$ has the added benefit of not being corrosive or phytotoxic to protected tomato plants and so can be used during cropping. Domestos Extended Power gave the best control and was the only product to eliminate all bacteria in the biofilms, but the doses recommended for surface disinfection have a much higher active chlorine concentration and are not suitable for use in-crop. Further evaluation will be needed to determine the minimal effective concentration of the Domestos formulation to assess potential phytotoxic and corrosive effects as well as the potential to cause chlorate residues in produce.

It was determined in Objective 4 that plants can be infected or clean at arrival on commercial sites. Both Carbon Gold and Vitix treated plants in Trial A (cv. Piccolo) at arrival tested negative for *R. radiobacter* and the Ri plasmid, but the vast majority of these treated plots became infected with root mat disease by the end of the season. This suggests infection arose from a pre-existing inoculum source on Site A, most likely as a biofilm in the irrigation piping. This underlies the importance of effective disinfestation of the glasshouse at crop turnaround. Comprehensive clean-up and the introduction of clean, uninfected plants from propagators should allow for the elimination of root mat disease on-site. Results from this study would recommend the use of a chlorine-dioxide based product such as Clorious$_2$ during cropping, and the use of Domestos Extended Power in treating surfaces in the absence of plants during cropping and at crop turnaround as the most effective way of managing *R. radiobacter.*

Conclusions

- Fera Science Ltd. are in possession of a rapid molecular assay capable of confirming the presence of pRi T-DNA in plant roots.
- Seed and irrigation water sampled from the two Dutch propagators providing plants for the three trials all tested negative for *R. radiobacter* and the pRi.
- 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.
- No treatment was successful in eliminating root mat disease in tomato, however several reduced incidence and / or severity of symptoms.

- 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.
- Vitix increased the incidence of the disease nearly three-fold compared to the untreated in Trial A when applied in propagation alone, suggesting treatment at propagation is an important component of disease management.
- Infection in Trial B likely occurred from pre-existing inoculum 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 suggest the irrigation system, and possibly levels of dissolved oxygen, may play a role in disease occurrence.
- Cultural practices such as the application of additional coir to severely infected slabs, addition of extra irrigation pegs, application of routine calcium sprays and generative steering may in part restore a crop to a more manageable status.
- Chlorine-based biocides were significantly more effective than hydrogen peroxide-based biocides at killing *Rhizobium radiobacter* at the concentrations and exposure times tested.
- 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.
- The chlorine dioxide-based biocide (Clorious$_2$) 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.

Knowledge and Technology Transfer

- Presentation to the Tomato Study Group, Isle of Wight, 20 June 2017 (D Kaye)
- Presentation to the 2017 Tomato Growers Association, 21 September 2017 (D Kaye)
- Presentation to the Tomato Working Party, Red Roofs Nursery, 11 January 2018 (D Kaye)
- Article in AHDB Grower magazine, (in preparation).

References

Miller, T. D., Schroth, M. N. 1972. Monitoring the epiphytic populations of *Erwinia amylovora* on pear with a selective medium. Phytopathology*,* 62, 1175-1185.

Bosmans, L., Moerkens, R., Wittemans, L., De Mot, R., Rediers, H., Lievens, B., 2017. Rhizogenic agrobacteria in hydroponic crops: epidemics, diagnostics and control, Plant pathology, 66**,** 1043-1053.

Berendson, R., Pieterse C., Bakker P., 2012. The rhizosphere microbiome and plant health, Trends in Plant Science, 17, 8 478-486

Lelliott, R.A. and Stead, D.E. 1987. Methods for the diagnosis of bacterial diseases of plants. Blackwell Scientific Publications, Oxford, UK. 216 pp.

Weller, S.A. and Stead, D.E. 2002. Detection of root mat associated *Agrobacterium* strains from plant material and other sample types by post-enrichment TaqMan PCR. J. Appl. Microbiol. 92, 118-26.

Weller S.A., Stead D.E., Young JPW. 2006. Recurrent outbreaks of root mat in cucumber and tomato are associated with a monomorphic, cucumopine, Ri-plasmid harboured by various Alphaproteobacteria. FEMS Microbiology letters 258**,** 136-43

Glossary

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

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

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

Quorum sensing – a signalling system between bacteria

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

Appendices

Appendix 1 – Treatment list and Trial plans for Trials A, B and C

Treatment list

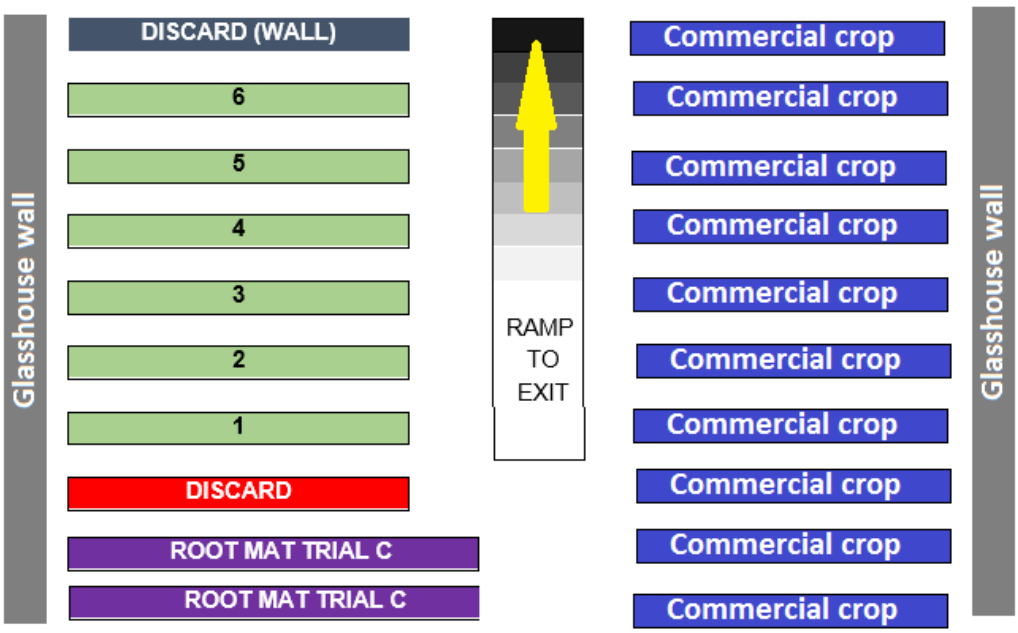
Treatment no.	Product	Treatment timing	
		Propagation	Crop
1	Untreated	×	×
2	Untreated	×	×
3	Carbon Gold	✓	×
4	Carbon Gold	✓	✓
5	Vitix	✓	×
6	Vitix	×	✓
7	Vitix	✓	✓

Trial plan for Trials A, B and C

Block	Plot	Trial A		Trial B		Trial C
1	1	6		2		6
1	2	3		4		7
1	3	2		3		2
1	4	4		6		3
1	5	1		7		1
1	6	5		5		4
1	7	7		1		5
2	8	3		1		6
2	9	2		5		1
2	10	5		6		2
2	11	7		7		5
2	12	6		3		3
2	13	4		2		7
2	14	1		4		4
3	15	2		1		3
3	16	5		6		6
3	17	1		5		1

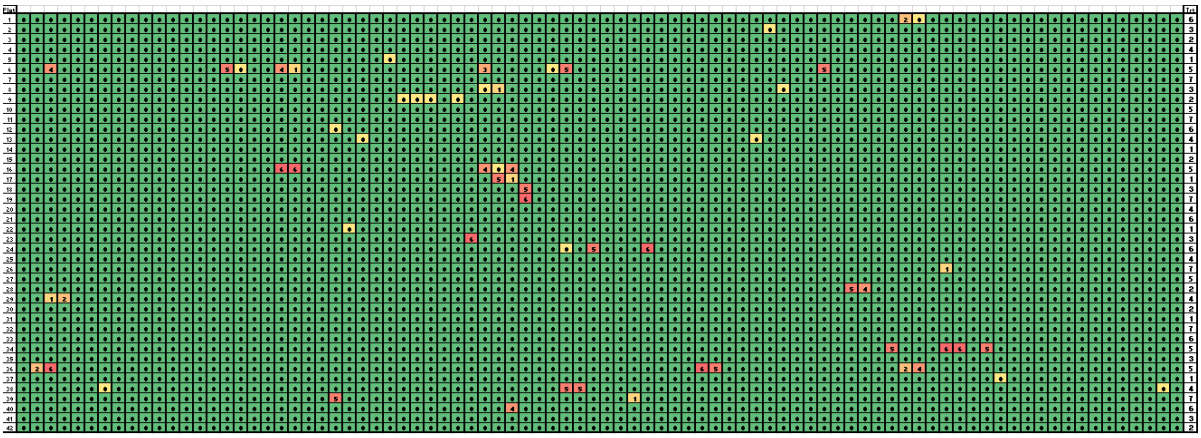
3	18	3		2		5
3	19	7		7		2
3	20	4		4		4
3	21	6		3		7
4	22	1		3		3
4	23	3		6		2
4	24	6		5		5
4	25	4		7		7
4	26	7		1		4
4	27	5		2		6
4	28	2		4		1
5	29	4		6		5
5	30	2		4		2
5	31	1		3		7
5	32	7		5		1
5	33	6		1		3
5	34	5		7		6
5	35	3		2		4
6	36	5		7		2
6	37	1		3		6
6	38	4		2		4
6	39	7		1		1
6	40	6		6		3
6	41	3		4		7
6	42	2		5		5

Appendix 2 – The six additional rows of treated Vitix assessed at Site 2, next to Trial C

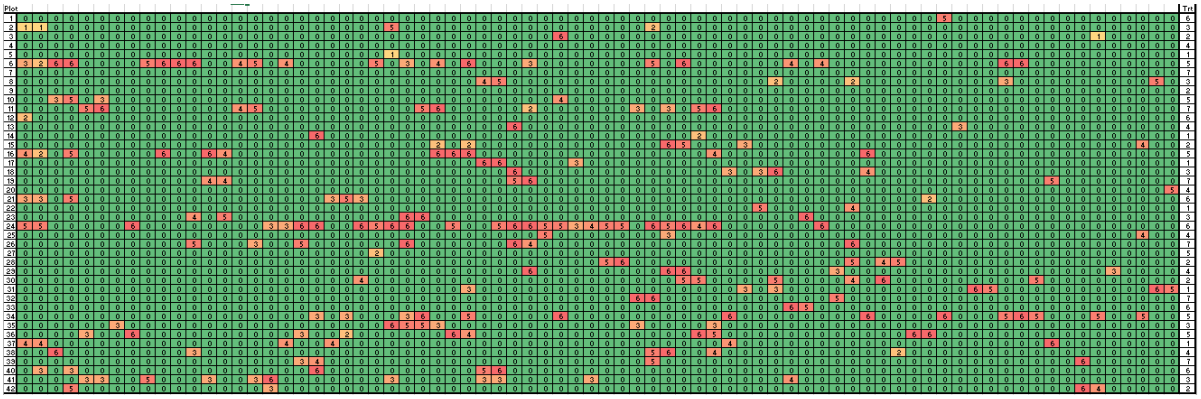


Appendix 3 – Heat maps for Trials A, B and C at the first symptom and end-of-season assessments showing the distribution and severity of root mat disease over time.

Trial A – First-symptom assessment, May 2017

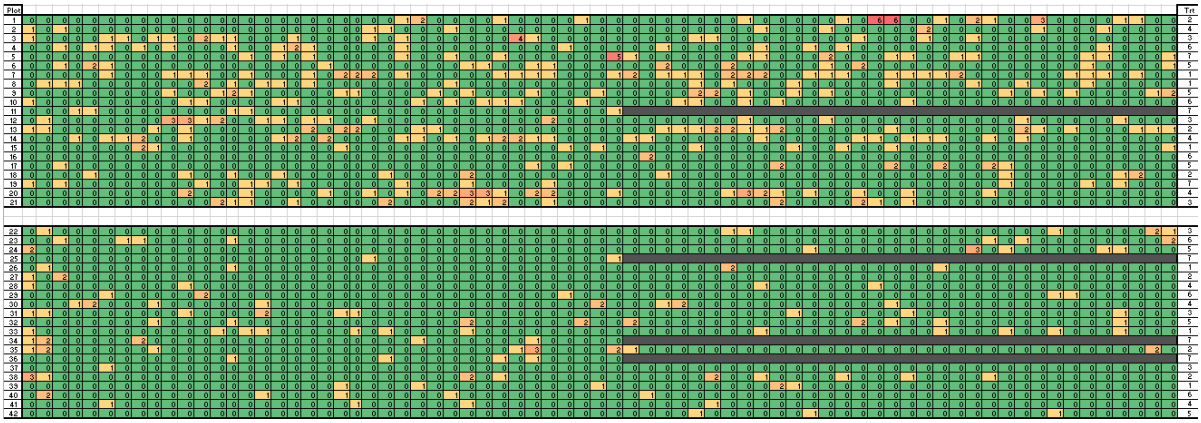


Trial A – End-of-cropping assessment, November 2017

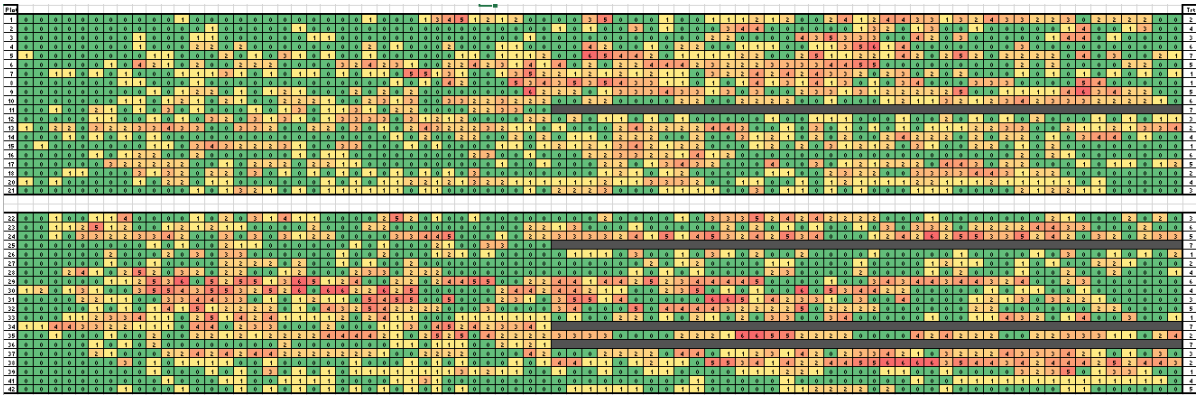


Green cells represent no infection whilst yellow to red correspond to infected plots with greater levels of severity. Yellow = low, one to two roots. Red = high, 50%+ of propagation cube surface affected / swelling of propagation cube

Trial B – First-symptom assessment, June 2017

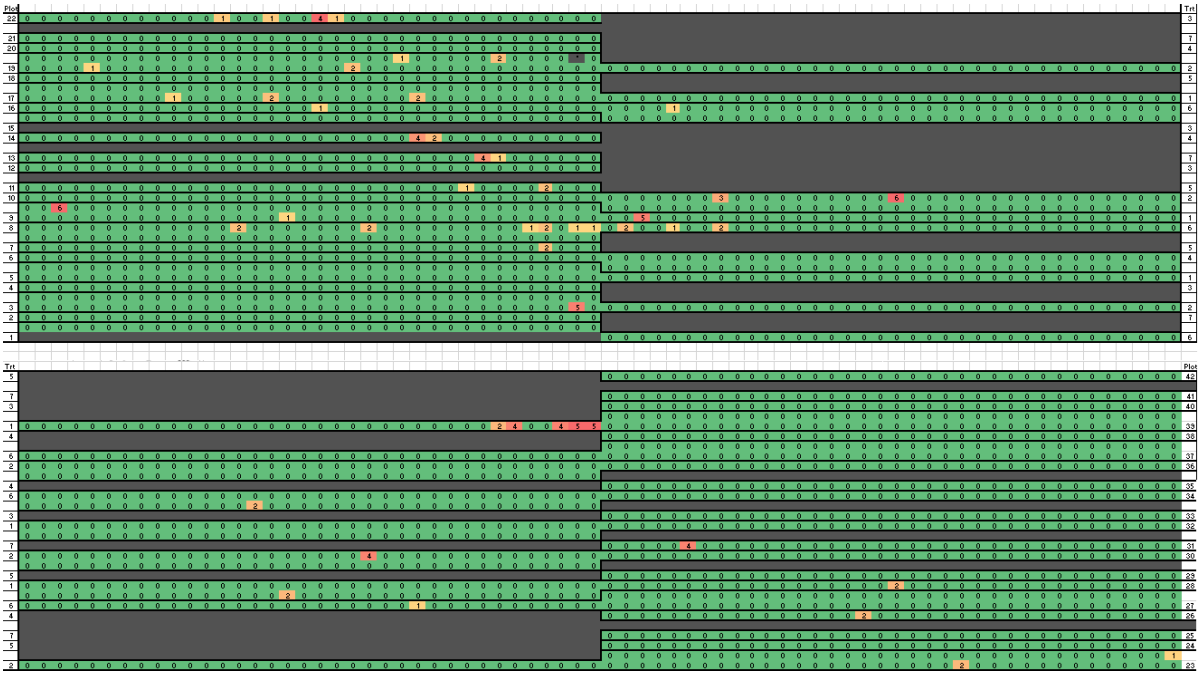


Trial B – End-of-cropping assessment, November 2017



Green cells represent no infection whilst yellow to red correspond to infected plots with greater levels of severity. Yellow = low, one to two roots. Red = high, 50%+ of propagation cube surface affected / swelling of propagation cube
Grey cells correspond to areas of the crop not included in the trial and not assessed

Trial C – First-symptom assessment, June 2017

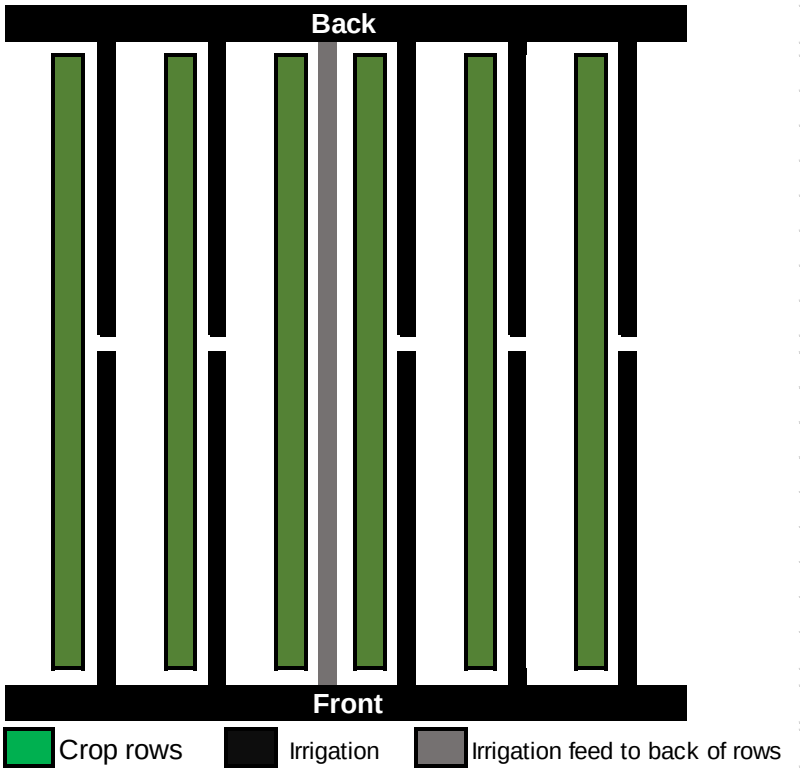


Trial C – End-of-cropping assessment, November 2017

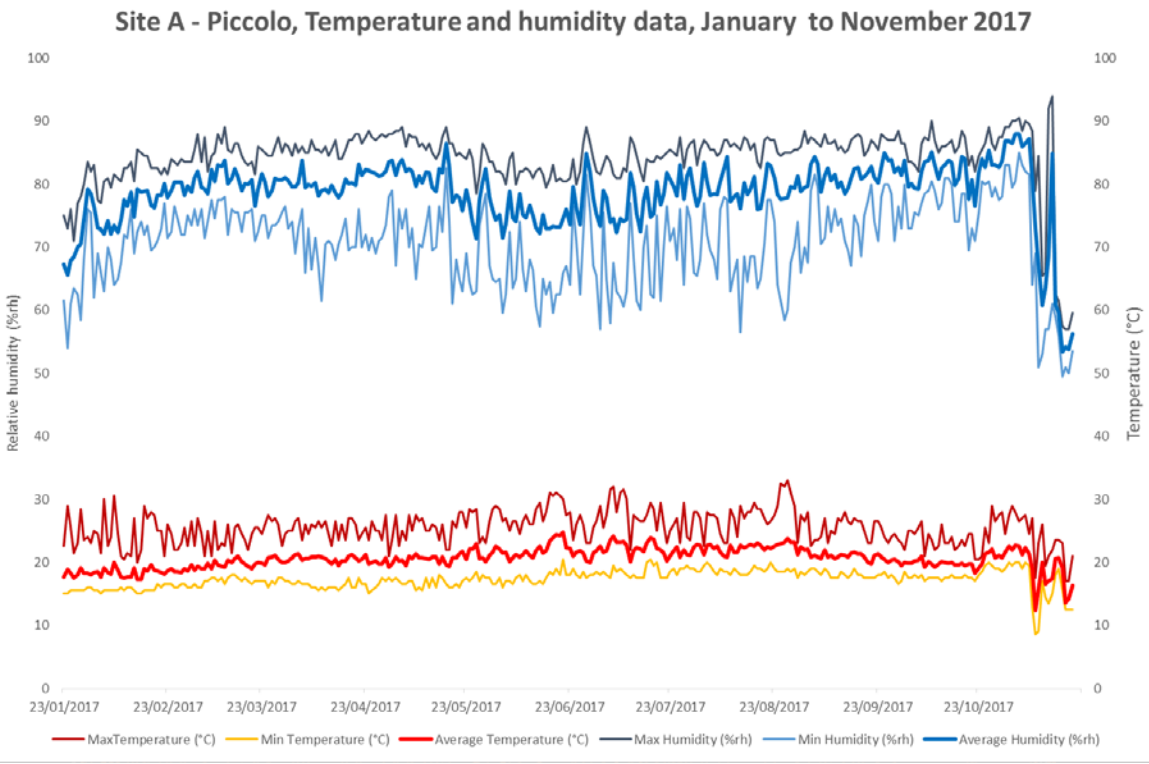
Green cells represent no infection whilst yellow to red correspond to infected plots with greater levels of severity. Yellow = low, one to two roots. Red = high, 50%+ of propagation cube surface affected / swelling of propagation cube
Grey cells correspond to areas of the crop not included in the trial and not assessed

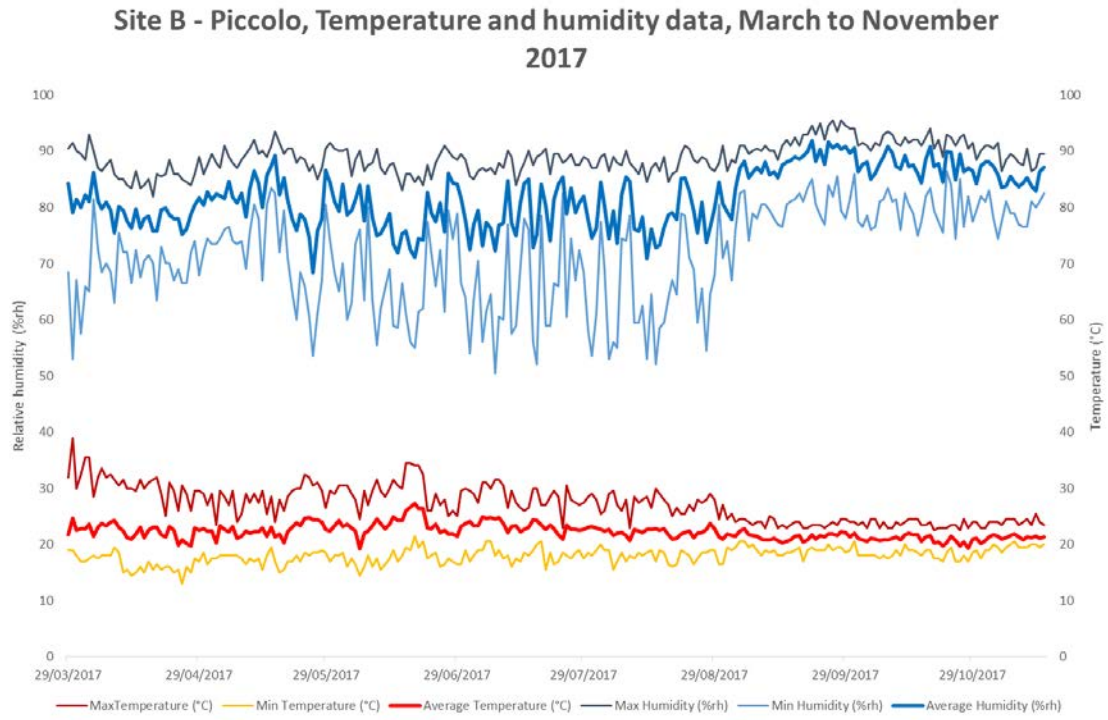
Appendix 4 – Irrigation plan of additional Vitix treated area at Site 2. Irrigation water flows to the back of the rows via a central pipe (grey) before splitting and feeding back into each row.

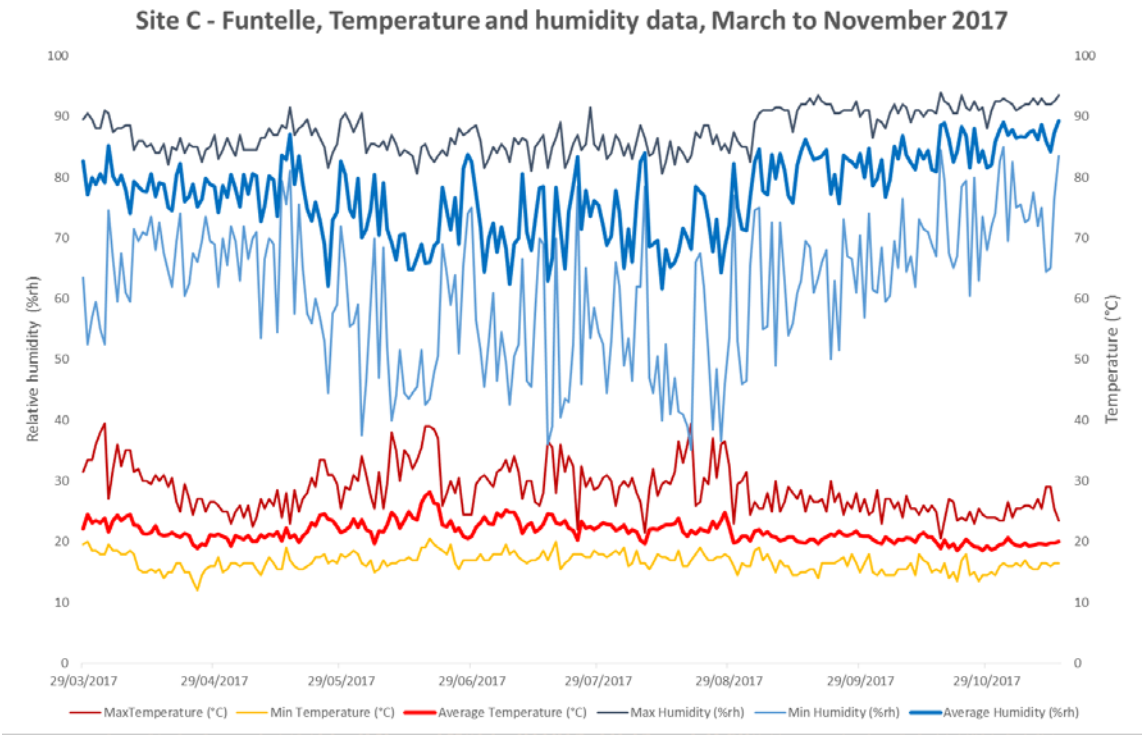
The six rows monitored in this house, in addition to Trial C, were all located on the same irrigation system.



Appendix 5 – Temperature and humidity graphs for Trials A, B and C from planting to the trials conclusion, January-November (Trial A), March to November (Trials B & C).







N.B. Data loggers were placed ~15cm above the cube surface to give an indication of the root zone environmental conditions in the middle of the crop. The string supporting the logger placed in Trial C was found broken with the logger resting on the floor of the glasshouse compartment. It is not possible to determine at what time during the growing season this occurred. As such environmental conditions for Trial C cannot be relied upon to provide an accurate representation of cube conditions.

Appendix 6 – Additional variety observations

Several additional tomato varieties, grown on once-used slabs with a history of the disease, were observed at Site 2 from mid-Summer onwards. These were visually inspected for root mat disease and secondary consequences of the disease, including blossom end rot (BER). These varieties included a mixture of generative and vegetative varieties including Lyterno, Roterno, Elegance, Sweetelle and Vesuvius. Disease management practices, including coir supplementation on heavily infected slabs, supplementary irrigation and the use of calcium sprays to reduce BER were performed by the nursery staff and their general effectiveness discussed with the host grower. It is important to note that these are observations only, and not outcomes from a statistically robust trial. The observations provide insights into potential disease management strategies which may provide a benefit to commercial tomato growers, with work would be required to confirm their effectiveness.

At the date of the mid-crop assessment several of the varieties struggled with severe infections of root mat exhibiting considerable cube and slab coverage of root mat symptoms (Figures 34 and 35). This resulted in difficulty in managing and steering these crops.

Maintaining uniformity became difficult as water ran off the surface of the coir slabs not being absorbed. This often led to the development of BER (Figures 36 and 37), and a reduction in marketable fruit. Any fruit left rotting on the vine became infected with secondary infections such as penicillium and attracted unwanted pests which could lead to additional pest and disease issues (Figures 33 and 34).

In order to prevent irrigation run-off the coir bags were opened and additional coir was added on top of the existing root-matted material (Figure 34). Additional drip pegs were also introduced to the slabs to improve irrigation. This appeared to have a positive effect improving water management and the crops recovered to some degree by the end of the season. Routine calcium sprays were applied to the crops to reduce the effects of BER in fruit. Differences were also noticed between varieties with Lyterno and Roterno performing worst in the once-used infected slabs, suggesting possible differences in varietal susceptibility. Due to the plants arriving late at Site 2 they were steered to be generative from the start which may have helped to reduce root mat severity as it is generally believed that more vegetative varieties suffer worse from root mat disease. These practices improved the crops at Site 2 demonstrating it may well be possible to improve a crop heavily infected with root mat over time.

Figure 34 – A coir slab with severe surface root mat coverage. Note the additional irrigation peg placed in the centre of the slab to increase water absorption by the coir. Root mat symptoms have developed at this irrigation point which may lead to irrigation water running off the slab without being absorbed, or even blocking of the irrigation peg itself.

Figure 35 – A heavily infected slab with additional coir added on top of existing matted root to aid water absorption in cv. Roterno. Although this provides some benefits to the irrigation new root mat symptoms have developed on the surface of the additional coir. Dependent on the time of application further additional coir may need to be reapplied.

Figures 36 & 37 – Examples of blossom end rot in cv. Elegance at the mid-crop assessment in July reducing the proportion of harvestable marketable fruit available. Note the secondary fungal infections on several fruit. Additional pests were also associated with these rots.