ROOT MAT IN TOMATO

ANNUAL REPORT PC 241

To:

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Protected hydroponic tomato: investigating the potential for various novel non-chemical techniques for the suppression or control of root-mat disease

April 2008

Project Title:	Protected hydroponic tomato: investigating the potential for various novel non-chemical techniques for the suppression or control of root-mat disease.
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Previous work:	PC 149 Cucumber & Tomato – investigation of the cause, epidemiology and control of root proliferation ('root-mat') in hydroponic crops. Defra HH2308SPC – Improved control of novel *Agrobacterium*-induced diseases in hydroponic crops through risk assessment and biological controls.
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The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiments were carried out and the results obtained have been reported with detail and 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 application.

AUTHENTICATION

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

Signature ……

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Project Manager
Stockbridge Technology Centre

Date ……

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Headline

The effect of various slow sand and rock wool filters on root mat establishment and symptom expression was not determined and whilst no root mat symptoms were observed on tomato grafted onto an aubergine root-stock, yield was severely reduced. However, a small trial glasshouse indicated Gliomix or Biomex SA applications may prevent root mat symptoms developing.

Background and expected deliverables

Root mat was first reported in the UK in the 1970s on soil and straw bale grown cucumbers. The disease causes massive over-production or proliferation of roots on affected plants which ultimately results in increased vegetative growth of the foliage. This can cause many problems in terms of crop management and poor crop quality. By the end of the 1970s the disease had disappeared in soil and straw bale crops. Root mat re-appeared in hydroponic cucumbers in 1993. Outbreaks continue, though both incidence and severity in cucumbers has reduced in recent years. This may be due to the reduction in the number of infected but symptomless plants in propagation, or perhaps a change to increase the number of crops produced each year, which makes management of infected crops much easier.

The disease has now occurred in tomato crops where it has persisted at a severe level in hydroponic crops on some nurseries in the UK. There is a natural concern that it could spread to infect further nurseries in the future. As there is a current lack of proven effective control measures root-mat poses a significant potential risk to economic production of tomatoes throughout the UK. It has been estimated that losses due to root-mat in tomato are currently in the region of £0.75M/annum for one company alone (caused by an increase in secondary disease and crop management costs) though the potential for greater loss is considerable if the disease spread more widely to other tomato nurseries.

Figure 1. Tomato with severe root mat symptoms

Chemical ‘disinfection’ strategies have so far failed to control the disease. A recently completed Defra project (HH2308SPC) indicated that increasing the microbial diversity within the rockwool growth substrate led to a suppression of root-mat symptoms in hydroponic cucumber crops. Also observations on commercial nurseries where a natural decline of root-mat symptoms occurred over a number of seasons in biologically diverse organic soil-grown cucumber and tomato crops support this hypothesis. This led to the possibility that increasing the population of naturally-occurring microbial antagonists might suppress or prevent the development of root-mat commercially. This study has therefore looked at a number of alternative, non-chemical strategies to try and minimise or eliminate the risk of root mat in hydroponic tomatoes. It is hoped that results from this investigation, when complete, can be applied to commercial glasshouse production to provide a successful and cost effective method of controlling root mat disease.

Summary of the project and main conclusions to date

Four primary objectives were set:

a) To evaluate the potential of different filtration techniques based on the principle of slow sand filtration (SSF), but incorporating organic substrates including soil, to mimic the disease suppressive effects observed commercially in organic tomatoes.
b) To investigate the impact of formulated (non-regulated) microbial preparations on root-mat through increased microbial diversity.
c) To investigate the potential of grafting onto alternative rootstocks e.g. Aubergine as a means of suppressing or preventing root-mat in tomatoes.
d) To determine whether the principle of cross-protection, as it applies to other pathogens is effective against root-mat of tomatoes.

Work on objectives a), b) and d) was initiated in the first year of this project (2006), further work on these objectives and objective c) was carried out in 2007 and will be continued in 2008.

<u>Objective a</u>: Investigating the possible effects of slow sand filtration techniques on root-mat in tomatoes

Year 1 (2006)

A small-scale semi-commercial hydroponic tomato crop cv Claree was grown at STC and was irrigated using re-circulation system which had passed through the following 6 modified slow sand and rockwool filters.

1. Inoculated – conventional slow sand filter (SSF)
2. Inoculated – slow rockwool filter (SRF)
3. Inoculated – SSF + organic soil 'sandwich'
4. Inoculated – SRF + organic soil 'sandwich'
5. Inoculated – SSF + soil/straw 'sandwich'
6. Inoculated – SSF with soil/straw throughout filter.

Plots acting as positive and negative controls were included using a standard run-to-waste system. The trial was inoculated using material collected from severely affected roots exhibiting root-mat which was isolated from Claree in 2006.

The crop established well, however, despite two inoculations of the crop, satisfactory symptom expression did not occur in the inoculated control plants. A few very early root-mat like symptoms were observed in the inoculated control plots, however the symptoms did not develop further.

Year 2 (2007)

We had hoped to repeat the above work refining the most promising filter set-up to validate the year 1 results. However, the lack of good symptom expression in 2006 meant that we needed to repeat the work with slight modifications as agreed at the project review meeting. In 2007 2 tomato cultivars – Claree and Elegance were used together with two cucumber plants, cv. Aviance per plot as previous experience at CSL had shown symptom expression, following artificial inoculation, to be stronger in cucumbers than tomatoes and these plants would therefore act as a root-mat 'indicator' in theory, at least. Artificial inoculations were carried out on the crop when the plants were young to try and aid infection. However, once again symptoms failed to develop strongly despite early signs of infection in the crop.

The lack of symptom expression is both intriguing and frustrating. It is likely that factors affecting symptom expression are highly complex, influenced not only by the presence of the pathogen, but also by other biotic and abiotic factors.

During the same period it was reported that root-mat had occurred at a tomato nursery in the north of England for some time, and that it was quite severe in 2007 so the absence of root-mat symptoms was clearly not a result of local environmental factors. It was agreed that work in 2008 should focus attention on studies on commercial nurseries at both the known affected sites to help ensure that some useful data and information could be gathered. Work was already in progress to construct a slow sand filter at the southern site and this would then be in place for the 2008 crop.

Objective b: Evaluating formulated microbial products for root-mat control in tomato

Year 1 (2006)

A series of small-scale experiments involving the application of a range of commercially available microbial products were carried out on inoculated young tomato plants cv. Claree at CSL during 2006. As with the glasshouse trial at STC very poor symptom expression was seen and few conclusions could be drawn except that in molecular tests the causal pathogen (*Agrobacterium)* was found to be present in the roots of all plants with the exception of those treated with Gliomix and the negative (non-inoculated) control plants following molecular analysis eight weeks post-inoculation.

Year 2 (2007)

Following the disappointing results seen in Yr 1, the work was repeated using cucumber plants instead due to their apparent propensity to produce root-mat symptoms using a modified range of microbial products.

Details of Bio-control products under evaluation in 2007

Treatment	Manufacturer	Active ingredient or	Rate of application

		organism	(per 500ml)
1. Uninoculated (negative) control	-	-	-
2. Inoculated untreated (positive) control	-	-	-
3. Seasol	Seasol International	Bull kelp concentrate	1.7ml
4. Biomex SA	Omex Agriculture	*Trichoderma* spp.	0.5ml
5. FZB	Omex Agriculture	*Bacillus spp.*	0.25ml
6. Garshield	Garlic Farms	Garlic Extract	50µl
7. Gliomix	Fargro Ltd	*Gliocladium* sp.	1g
8. GLD	Omex Agriculture	Garlic extract and salicylic acid derivative	50µl
9. PHC Complete Plus	Fargro Ltd	Rhizobacteria, *Trichoderma, Gliocladium*, Yucca Extract	0.655g
10. Stimagro	Fargro Ltd	*Streptomyces* sp.	0.25g

Symptoms were observed on the untreated inoculated plants, and by the end of the experiment only plants receiving applications of either Gliomix or Biomex SA (and the negative control plants) were free of root-mat symptoms.

The work will be repeated in tomatoes in 2008 using an isolate *Agrobacterium* recovered from symptomatic plants in a commercial nursery in November 2007 and in at least one commercial crop in 2008.

Proportions of plants confirmed infected with causal genetic material and showing root-mat symptoms at 5 and 16 wks after inoculation.

Treatment	Proportion of plants infected with causal genetic material		Proportion of plants with symptoms	
	18 May	3 August	Cube	Slab
1. Positive control	2/4	4/4	4/4	0/2
2. Negative control	0/4	4/4	0/4	0/2
3. Biomex SA	2/2	2/2	0/2	0/1
4. Garshield	2/2	2/2	2/2	1/1
5. GLD	2/2	2/2	1/2	Possible
6. Seasol	1/2	2/2	0/2	1/1
7. PHC Compete	2/2	2/2	1/2	0/1
8. Gliomix	2/2	2/2	0/2	0/1
9. FZB	2/2	2/2	1/2	Possible
10.Stimagro	2/2	2/2	2/2	1/1

Objective c: Evaluation of the potential of grafting onto alternative root stocks as a means of suppressing or preventing root-mat in tomatoes

Year 2 (2007)

The tomato cultivar Jack Hawkins was successfully grafted onto the aubergine root stock Madonna by a commercial propagator in 2007. The plants were planted at a commercial nursery with a history of root-mat in the South of England. Although no root-mat developed on the plants during the season it was also reported that the incidence of root-mat on rockwool crops across the nursery was dramatically reduced compared to previous years. This factor is intriguing and may suggest that a build up of naturally suppressive organisms or a natural weakening of the pathogens virulence may be occurring. In terms of this experiment it unfortunately meant that no firm conclusions could be drawn as to the efficacy of the aubergine rootstock. However it was observed that the graft combination impacted in a very negative way on yield, reducing it by approximately 80% - this alone would result in this potential solution being unacceptable unless a significantly vigorous aubergine cultivar could be found.

Objective d : Evaluating the principle of cross-protection for root-mat control in tomato

Year 1 (2006)

Observed differences in the severity of root-mat symptoms at two commercial nurseries and subsequent testing have shown that all the *Agrobacterium* isolated at nursery A contain different genetic material than at nursery B. This suggests a correlation (in this instance) between genetic material type and symptom severity. Work was carried out to investigate whether inoculation with *Agrobacterium* containing the less virulent genetic material would provide any protection when the plants were challenged at a later date with the more virulent material. Unfortunately, a lack of symptom throughout all the treatments again meant that no firm conclusions could be drawn during the experiment.

Follow-on work

This study is being repeated in glass-house tomato crop at STC during early 2008 using a range of collected *Agrobacterium* isolates. It is too early to comment on results in this annual report.

Financial benefits

Root mat is a serious root disease of tomato and cucumber that interferes with the normal root production of the host allowing it to proliferate uncontrolled. This affects plant physiology, crop management, and susceptibility to other pathogens, yield and overall fruit quality. In addition to the direct impact of root mat the indirect effect of secondary pathogens, especially *Pythium* and *Botrytis*, can also be very important not only because of the direct commercial loss but also because of the need for fungicide intervention. Increased use of pesticides conflicts with the overall pesticide minimisation 'goals' of the Tomato Growers Association. It has been estimated that losses due to root mat in tomato were in the region of £0.75M/annum though the potential for greater loss is considerable now that the problem is known to occur on other tomato nurseries in the UK. There is therefore a significant financial incentive to identify and implement effective control measures for this pathogen before it becomes more widespread throughout the UK.

Action points for growers

Continue to monitor crops for symptoms of root mat and alert the Project Leader to any unusual symptoms or new developments.

Project Co-ordinator Comments

"Root Mat continues to be a significant problem in hydroponic crops in the south of England. In 2006 approximately 50% (around 10ha) and 2007 around 8ha of tomato crops were affected by the end of the season. Annual levels of root mat are variable and in 2008, despite high levels of bio-security on all sites we expect problems to persist as they have for the past 8 years.

Water is chlorinated, pathways regularly cleaned and a strict turn round policy and procedure been tuned to address the issue of root mat. Nevertheless, only in organic crops has a significant reduction in symptoms year on year been seen. Growers have tried various practical methods to deal with the problems caused by symptoms including increasing the volume of rockwool available to the affected roots and removing the plastic from rockwool slabs.

It is not only the presence of root mat, and the extra cost of additional management time which is cause for continued concern but also the economic loss, caused by reduced yields and fruit quality, which can be directly attributable to the extensive and seemingly uncontrollable spread of infection. Secondary infections of Pythium *and* Botrytis *contribute to an overall estimated 5-7% reduction in yield after infection manifests".*

Dr Phil Morley, April 2008

"Outbreaks of root-mat continue, although incidence and severity in cucumbers has reduced in recent years, partly because of action taken to reduce the problem in propagation and also because of the change to have up to three crops of shorter duration that prevents the problem building up in any one crop. The situation seems to be getting worse in tomato crops with two nurseries suffering quite severe infection. One of the problems caused by the root proliferation is the reduction in water penetrating into the growing media thus reducing crop potential.

It is important to remember when dealing with this problem that the Agrobacterium can be easily dealt with by steam sterilisation of the growing media but the plasmid that causes the problem is not destroyed by such steam sterilisation.

The plasmid left behind in the growing media can then be picked up by Agrobacterium that can re-invade the growing media the following season. Extensive sterilisation and disinfection across nurseries has never successfully eradicated the disease and therefore will allow the problem to be carried over from one season to the next. Infested growing media should not be re-used – even if steam sterilised.

What is needed to combat the problem is a method that out competes the Agrobacterium or that removes the plasmid from the system."

Derek Hargreaves, April 2008

SCIENCE SECTION

Introduction

Root-mat was first reported in cucumbers in the UK in the 1970s. The disease persisted for several seasons then disappeared from commercial crops even though there had been no specific intervention to control the problem. The symptoms re-surfaced in hydroponically-grown cucumber crops in 1993. The disease has persisted in several nurseries since then. In 2008 it was observed for the first time in Dutch-raised cucumber plants grown in the UK. Of considerable concern is that in the last nine years it has also appeared in large-scale hydroponic commercial tomato crops. Root-mat is characterised by an over-proliferation of roots. The development of the extended root system affects the plant physiology, increasing vegetative growth in the aerial parts of the plant, making crop management (where some plants are affected; others not) very difficult. Fruit quality can also be affected and the susceptibility of the crop to other pathogens such as *Pythium* and *Botrytis* has been seen to increase.

Figure 2. Tomato roots severely affected by root-mat

The symptoms are caused by a small circular DNA element (the Ri- plasmid). On infection, a piece of this plasmid (T-DNA) is transferred from the vector *Agrobacterium* to the root cell where it is incorporated into the root cell nucleus. Genes encoded on the T-DNA are expressed in transformed roots causing root proliferation (Figure 2). This stimulates the roots to produce an opine compound (cucumopine) which in turn provides a nutrient source for *Agrobacterium*. Previous studies sponsored by the HDC (PC 149) and Defra (HH2308SPC) have focused mainly on investigating and controlling the problem in cucumbers. Evidence collected during these

studies suggests that increasing the microbial diversity in the rockwool slabs (and glasshouse environment) may be important in reducing root-mat symptoms perhaps through direct competition or antagonism. This concept has been developed further following commercial observations that root-mat symptoms reduced season by season in an organic soil-grown tomato crop without specific intervention, leading to the suggestion that naturally occurring antagonists were, in some way, out-competing the root-mat pathogen.

Although root-mat is still present in commercial cucumber crops, in many cases, the problem has been alleviated. This potentially might be due to reduction in the number of infected, but symptomless, plants in propagation or perhaps a change in cropping practices e.g. by increasing the number of crops/season. This decreases the time by which individual plants are affected by the disease, hence alleviating symptom expression and therefore making management of the crop easier.

The investigation reported here focused on root-mat in long-season tomatoes where adjustments to cropping frequency are not economically viable and a single, long-term crop is grown each season. The ultimate aim of the project is to find a practical and economic solution to root-mat for the UK tomato industry, through the use of one or more non-chemical intervention strategies.

This study has four primary objectives:

a) To evaluate the potential of different filtration techniques based on the principle of slow sand filtration, but incorporating organic substrates including soil, to mimic the disease suppressive effects observed commercially in organic tomatoes.
b) To investigate the impact of formulated (non-regulated) microbial preparations on root-mat through increased microbial diversity.
c) To investigate the potential of grafting onto alternative rootstocks e.g. Aubergine as a means of suppressing or preventing root-mat in tomatoes.
d) To determine whether the principal of cross-protection, as it applies to other pathogens is effective against root-mat of tomatoes.

Work on objectives a), b) and d) was initiated in the first year of this project (2006) and further work on these objectives and also on objective c) was carried out in 2007. Separate elements of the work in 2007 will be carried out at Stockbridge Technology Centre (STC), the Central Science Laboratory (CSL) and, in later parts of the study, on commercial tomato nurseries in northern and southern England with support from ADAS and STC.

Methods & Materials

<u>Objective a</u> : Investigating the possible effects of slow sand filtration techniques on root-mat in tomatoes

Work to investigate the possible effects of slow sand filtration techniques on root-mat was initiated at STC during the 2006. Following poor symptom expression in the trial during 2006 it was agreed that the trial would be repeated in 2007 but with specific modifications as agreed by the project team. A mixed crop, consisting of 2 cultivars of tomato – Claree and Elegance (alternating plants in each row) with 2 Aviance cucumber plants/row, was grown using a re-circulating hydroponic system using methods which followed near-commercial practice. It was hoped that a mix of tomato cultivars with the addition of cucumber plants as 'indicator' plants would increase the chances of good symptom development following artificial inoculation. Previous work on root-mat in cucumbers had led to the development of robust artificial inoculation techniques which unfortunately did not appear to have been successful when used on tomatoes in the 2006 STC crop.

The six filters which were constructed for the 2006 trial were drained down completely over the winter period as was recommended by Dr Tim Pettitt. Prior to re-instatement the entire fine sand layer was removed and replaced with new sand (plus any organic amendment). In the slow rockwool filters the top 40cm of rockwool was replaced and a 5 week priming period was carried out using lagoon water from STC. All other aspects of the trial design were replicated from the Year 1 study.

<u>Treatments</u>:

1) Uninoculated control – RTW
2) Inoculated control - RTW
3) Inoculated – conventional slow sand filter (SSF)
4) Inoculated – slow rockwool filter (SRF)
5) Inoculated – SSF + organic soil sandwich
6) Inoculated – SRF + organic soil sandwich
7) Inoculated – SSF + soil/straw sandwich
8) Inoculated – SSF with soil/straw throughout filter.

Crop Diary

16.3.07	Fine sand and rockwool layers replaced
22.3.07	Priming initiated
26.3.07	Tomato seed cvs. Claree and Elegance sown
18.4.07	Cucumber seed cv Aviance sown
3.5.07	Trial planted
11.5.07	1st root-mat assessment
15.5.07	Inoculation with isolate 6399 from Claree (2006) and 6338 from Aviance
18.5.07	Inoculation with isolate 6399 to Elegance tomatoes
26.6.07	2nd root-mat assessment

7.8.07 3rd root-mat assessment

30.8.07 4th root-mat assessment

1.11.07 5th root-mat assessment

9.11.07 Root samples collected and crop removed.

Inoculation methodology

Fresh root-mat infected roots from Claree and Elegance tomatoes was collected from crops in the affected nursery in southern England. Isolations on the root material carried out at CSL showed the *Agrobacterium* to be present and to be positive for the Ri-plasmid in the roots of both cultivars; however it proved impossible to produce single cultures of the isolates which were positive for the plasmid. After several attempts it was agreed that the isolate collected from Claree in 2006 (6399) would be used to inoculate both tomato cultivars as it was felt that the timing of the inoculation process should be as early as possible and additional attempts would result in delays. The isolate collected from Aviance cucumbers (6338) in 2006 was also used.

As in the previous year, a 10^6 cells/ml suspension in phosphate buffer of the *Agrobacterium* carrying the plasmid was produced. A 1 litre aliquot was poured into the top of each filter, whilst 10ml aliquots were directly applied to the respective plants in the 'direct inoculation' row in each plot (see Trial Plan in Appendix 1). Additionally in 2007, plugs of symptomatic roots of the two tomato cultivars, recently collected from affected crops, were inserted into the rockwool blocks of the respective cultivars in the 'direct inoculation' row. It was hoped that this would further enhance the chances of infection.

Growing methodology

The glasshouse was maintained at a day & night temperature of 19ºC with venting set at 21 ºC. Irrigation timing and frequency were carried out automatically via a Vocom system and was adjusted to fit the demands of the crop throughout the season. A concentrated feed solution was mixed automatically from separate A & B tanks using a standard tomato feed regime (as advised by Derek Hargreaves). This was automatically 'dosed' into the 'clean' tanks post-filtration. However because of the constantly fluctuating water levels in the tanks combined with the fact that solution returning to the 'dirty' tanks as run-off from the plots contained varying amounts of nutrition (depending on the weather conditions) it proved difficult to maintain a standard fertigation concentration. The electrical conductivity (EC) of the solution was monitored on a regular basis (every 2-3 days), the 'dirty' tanks were topped-up with fresh mains water and the EC was adjusted in both the clean and dirty tanks until a value of 3 – 4ms could be achieved. The uninoculated and inoculated control plots which did not include a slow sand filtration treatment used a run-to-waste system. Plots in the control treatments were irrigated with mains water which was fed via a double

Dosatron arrangement providing A & B feed solutions. The EC of the irrigation solution was monitored regularly and maintained at a similar concentration as the re-circulated rows.

Strict hygiene precautions were maintained in the glasshouse with restricted access, foot dips at both access points and alcohol sprays used on all monitoring equipment. Gloves were worn for any crop or filter work whilst work required on the crop e.g. twisting, side-shooting, harvesting was carried out on the untreated control plots prior to moving into the remainder of the crop.

Crop monitoring

During propagation, shortly before planting, some unusual surface root development was observed on a few of the cv. Claree plants and a larger number of the cv. Elegance plants. One plant of each cultivar which was showing symptoms, plus two other randomly taken samples, were collected and sent to CSL for Polymerase Chain Reaction (PCR) testing for the presence of rhizogenic *Agrobacterium* prior to planting.

An initial record was made of the number of plants showing the unusual roots on the block surface immediately post-planting and pre-inoculation. Subsequently, the crop was monitored on a regular basis for the development of root-mat symptoms throughout the duration of the trial. The 0-3 severity scale shown overleaf was employed during all the assessments. During the assessment carried out on the 26th June 2007, root cores were collected from 3 plants showing suspect root symptoms (sampled plants are highlighted on the relevant assessment sheet in Appendix 2). These samples were sent to CSL for PCR testing for the Ri-plasmid, a root core sample collected from another tomato crop at the STC site was also sent for comparative purposes.

Root samples were collected on the 9th November (25 weeks post-inoculation) and sent to CSL for testing for the presence of rhizogenic *Agrobacterium*. Four separate samples were collected from the 1st, 5th, 10th and 15th plant in each row. On receipt at CSL the samples were covered with sterile phosphate buffer and vortexed. 0.1mls of each suspension was added to 10 mls of an *Agrobacterium* selective Medium 1A broth. These broth cultures were incubated for 72 hours. At this time 0.1mls of the broth culture was removed and boiled for 5 minutes. These lysates were then used as templates for the *rol* real-time PCR which tests for pathogenic Ri-plasmid DNA

Regular monitoring of the flow rates from each filter was carried out throughout the duration of the trial[1]. As in 2006 following observed variability in filter flow rates it was decided that the filters would be 'limited' so that they all ran at as close to the slowest recorded rate as possible to try and

[1] Flow rate was measured in ml/min (Y) but has been converted to L/m^2/hr using the following formula:
$\frac{Y \times 60}{1000} \times 3$ (multiplication by 3 necessary as the surface area of the filters = approximately ⅓ metre).

maintain consistency between the filters. In general, flow rates for slow sand filters are considered to be suitable for the task at between 100 – 150L/m^2/hr.

A final assessment for root-mat was carried out following the removal of the crop on the 1st November. The root-mat symptoms were scored using the following 0-3 severity scale:

Root-mat assessment (0-3 severity scale)

0 – No symptomatic root development

1 – A few root-mat-like roots visible around the dripper area.

2 – Moderate amount of root mat roots over more of the block surface

3 – Large amount of root mat roots present – block swollen.

Objective b: Investigation into the control or suppression of root-mat in tomatoes using existing microbial products

Initial small-scale experiments on tomatoes at CSL in 2006 involving regular applications of a range of microbial products had shown a disappointing lack of root-mat symptoms resulting in an inability to draw firm conclusions regarding potential efficacy of the products under investigation.

The work was repeated in 2007, in the first instance on cucumbers[2] (with work on tomatoes to follow in 2008). The range of products tested are shown in Table 1 below.

Table 1: Details of Bio-control products under investigation in 2007

Treatment	Manufacturer	Active ingredient or organism	Rate of application (per 500ml) applied at 250ml/plant
1. Uninoculated (negative) control	-	-	-
2. Inoculated untreated (positive) control	-	-	-
3. Seasol	Seasol International	Bull kelp concentrate	1.7ml
4. Biomex SA	Omex Agriculture	*Trichoderma* spp.	0.5ml
5. FZB	Omex Agriculture	*Bacillus spp.*	0.25ml
6. Garshield	Garlic Farms	Garlic Extract	50µl
7. Gliomix	Fargro Ltd	*Gliocladium* sp.	1g
8. GLD	Omex Agriculture	Garlic extract and salicylic acid derivative	50µl
9. PHC Complete Plus	Fargro Ltd	Rhizobacteria, *Trichoderma, Gliocladium*, Yucca Extract	0.655g
10. Stimagro	Fargro Ltd	*Streptomyces* sp.	0.25g

Crop Diary for microbial study

- Seed sown on 6.3.07.
- First product inoculation on 20.3.07
- Second product inoculation on 28.3.07
- Third product inoculation on 3.4.07 (after plants transplanted to slabs)
- Inoculation with 10^8 cfu ml^{-1} *Agrobacterium* suspension (CSL 6338 & 6380) on 4.4.07.
- Fourth product inoculation on 5.4.07, thereafter weekly applications until end of experiment.
- Experiment terminated on 7.8.07.

Solutions of the products listed above were applied (250ml/plant) to 2 cucumber plants cv. Aviance per treatment two weeks after germination and, thereafter at weekly intervals. At four weeks post germination plants were inoculated with 5ml of a 10^8 cfu ml^{-1} of rhizogenic *Agrobacterium*

[2] The primary reason for conducting the initial study on cucumber was because of the greater level of confidence of securing symptom expression in this host following artificial inoculation.

suspension (Isolate 6338 from Aviance in 2006). Additional plants were either left uninoculated and untreated (negative control) or inoculated and untreated (positive control) for comparison purposes. Regular visual monitoring of the plants was carried out.

Objective c: Investigation into the potential of grafting onto alternative rootstocks e.g. Aubergine as a means of suppressing or preventing root-mat in tomatoes.

A batch (200 plants) of the plants of the tomato cultivar 'Jack Hawkins' was successfully grafted onto an aubergine rootstock (cv. Madonna) by a commercial propagator. The plants were planted in a trial row at the WS southern Nursery with known root-mat problems during the 2007 season. The crop was monitored during the season for the development of root mat symptoms and general agronomic performance by WS personnel.

Objective d: Investigation into the control or suppression of root-mat in tomatoes using cross-protection

An initial small-scale experiment was carried out on tomato cv. Claree at CSL in 2006. The trial used isolates of rhizogenic *Agrobacterium* collected from the WS southern nursery with a history of root mat. The two isolates had been shown to contain differing Ri-plasmid types which had resulted in differing severity of the symptoms *in vivo*. We hoped to investigate whether inoculation with a 'weaker' strain of the plasmid might offer some degree of protection against a later introduction of the 'stronger' or more virulent strain.

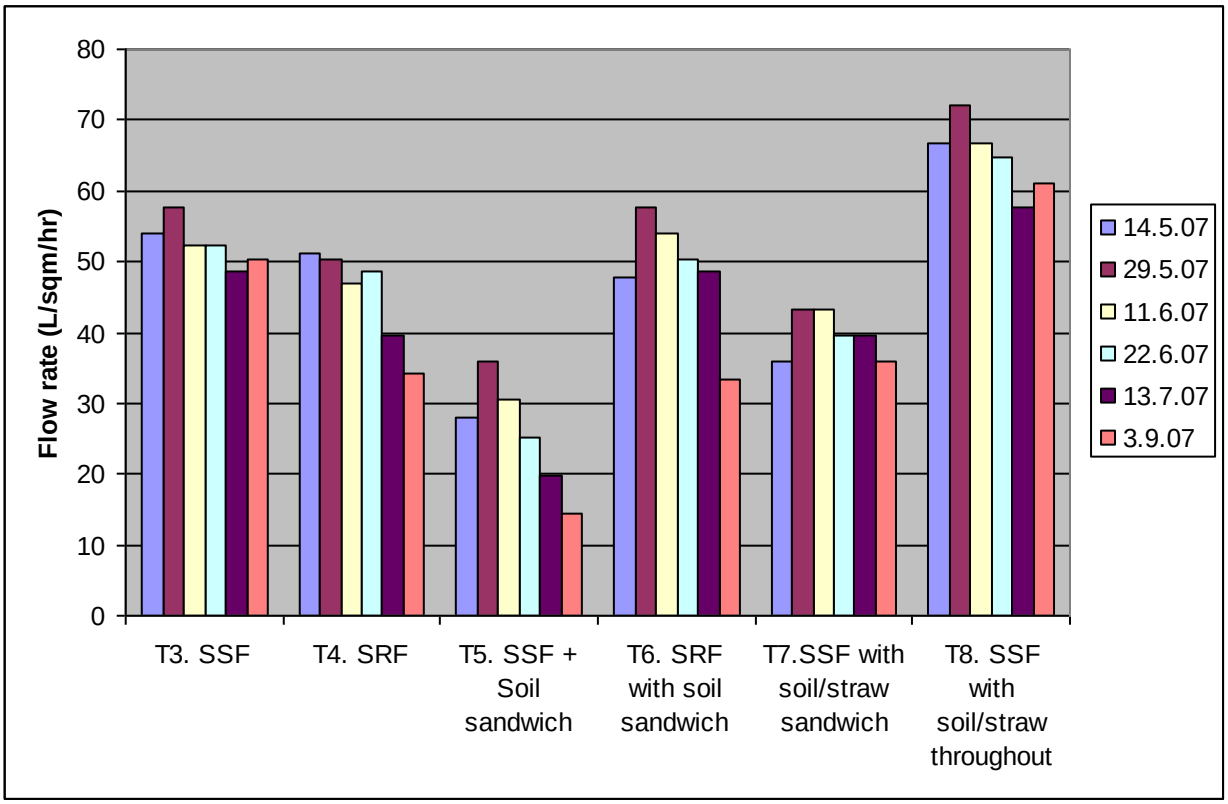
Symptoms were not observed on any of the inoculated plants during the 2006 CSL experiments. The work is being repeated, using a range of root-mat isolates, some of which have been collected from additional affected nurseries, in a small-scale glasshouse experiment at STC during 2008.

Results

<u>Objective a</u>: Investigating the possible effects of slow sand filtration techniques on root-mat in tomato

The plants established well and despite the mix of cucumbers and two tomato cultivars in the same trial area the crops grew well. The filters performed as expected and experience gained from Year 1 of the study was useful in Year 2 with regard to monitoring of flow rates and management of nutrition. As in 2006 the flow rates of the filters were limited during the trial period to as close to the rate of the slowest filter as was possible (Chart 1).

Chart 1. The recorded flow rates of the various filters over the trial period.



The initial PCR testing carried out on the propagated plants pre-planting showed that the plants which were showing unusual root symptoms on the blocks were <u>weakly</u> positive for the Ri-plasmid. Additional, randomly sampled, plants were negative. This result is of concern as it suggests that the plants may have become infected during propagation at STC. This may have been either via accidental contamination or by some, as yet undetermined method e.g. seed.

An assessment of the incidence of plants with unusual root symptoms was carried out immediately post-planting. A total of 33 plants exhibited abnormal surface rooting, of which 31 were of the cultivar Elegance. The details of this and the remaining assessments are shown in Table 2.

Table 2 . Showing the incidence and severity of root-mat symptoms in the STC glasshouse trial in 2007

Treatment	Propagation		Assessment dates											
	11.5.07*		26.6.07			7.8.07			30.8.07			1.11.07		
	Incidence		Incidence		Severity	Incidence		Severity	Incidence		Severity	Incidence		Severity
	E	C	E	C		E	C		E	C		E	C	
T1 Uninoculated control	6	1	0	0	0	0	0	0	0	0	0	0	0	0
T2 Inoculated control	7	0	10	0	0.52	4	0	0.14	3	0	0.09	1	1	0.09
T3 Conventional SSF	2	0	10	0	0.29	8	0	0.20	7	0	0.11	0	0	0
T4 Slow Rockwool Filter	6	0	0	0	0	1	0	0.02	0	0	0	0	0	0
T5 SSF with soil sandwich	3	1	7	3	0.32	1	0	0.02	0	0	0	0	0	0
T6 SRF with soil sandwich	2	0	5	1	0.18	7	0	0.20	6	0	0.04	2	0	0.04
T7 SSF with soil + straw sandwich	2	0	12	0	0.43	9	0	0.29	6	0	0.11	0	1	0.02
T8 SSF with soil + straw throughout.	3	0	1	0	0.02	1	0	0.02	3	0	0.01	0	0	0

Incidence = the number of plants out of a total of 44 in the 2 rows
Severity = 0-3 scale used – score is total across 2 treatment plots (i.e. not just plants with symptoms)
E = cv Elegance
C = cv Claree

* Possible root-mat like symptoms seen prior to inoculation

Full details of the various assessments are shown in Appendix 2 which allows tracking of the symptom incidence and severity of root symptoms on individual plants in the trial. Interestingly, even though cucumber plants were included as susceptible 'indicator' plants in this trial crop no symptoms were observed on any of the cucumber plants at any time during the trial period.

It is also relevant that the incidence of the root-mat symptoms recorded on the 26th June (6 weeks post inoculation) does not correlate with those recorded on the 11th May (immediately post-planting). It is evident that in the uninoculated control plots at least the early suspicious root-mat symptoms disappeared entirely (Table 2) and this suggests that the observed symptoms seen in propagation were not caused by root-mat. The symptoms may have been part of a normal growth characteristic for this cultivar, though; the weak PCR result does not necessarily support this. This may suggest that the sensitivity of the PCR test was sufficiently great to detect pathogenic *Agrobacterium* at levels below that required to induce symptoms.

The crop was inoculated with the rhizogenic *Agrobacterium* suspension and plugs of infected root material on the 15th (Claree & Aviance) and 18th May (Elegance) when the plants had been *in situ* for approximately 1 week. The first full assessment was carried out 6-8 weeks post-inoculation on the 26th June – six weeks post inoculation and was timed to coincide with the anticipated symptom expression which normally occurs around this time in inoculated plants. The incidence of plants showing symptoms had increased by this stage from the pre-inoculation assessment. Interestingly, none of the plants in the uninoculated control rows (T1) exhibited symptoms and this tends to suggest that the unusual surface root development observed during propagation was in fact not caused by *Agrobacterium* and the Ri-plasmid. By this stage, relatively high numbers of plants (especially in cv. Elegance) were showing unusual rooting in the inoculated control (T2), the conventional SSF (T3) and the SSF with soil + straw sandwich (T7) at this date. No symptoms were seen in the plants being irrigated via the SRF (T4).

At the 3rd assessment, 5-6 weeks later, the plants in the uninoculated control rows were still free from root-mat symptoms, and only 1 plant in the SRF (T4), SSF with soil (T5) and SSF with soil + straw (T8). The symptoms in the plants in T5 had reduced considerable (from 10 to 1 plant affected) since the previous assessment. Numerous plants in the Inoculated control (T2), SSF (T3), SRF + soil (T6) and SSF with soil + straw (T7) still showed root-mat-like symptoms at this time, though, the overall severity of these symptoms had reduced.

An overall decline in symptoms and severity was seen in the remaining assessments, and by the final assessment carried out on the 1st November, only 5 plants in the trial were showing root-mat symptoms (Figure 3). Two of these were in the inoculated control plot, 2 in the SRF + soil (T6) and

1 in the SSF with soil + straw (T7). However, as can be seen in the detailed assessment plans shown in Appendix 2, 2 of these affected plants had not been recorded with symptoms during the previous assessments. If good symptom expression had been apparent in the inoculated control (T2) plants, with symptoms remaining consistent and developing over time, this may have indicated that the disappearance of the symptoms over the trial period in the rows of plants being irrigated via the filters was due to biological and physical filtration of the rhizogenic *Agrobacterium* or, that metabolites from the filter micro-flora were eliciting the plant defence responses. However, once again, the lack of good symptom expression in the positive control plots does not allow us to substantiate this conclusion.

Figure 3. Root-mat symptoms on inoculated control plant (T2) at STC in 2007

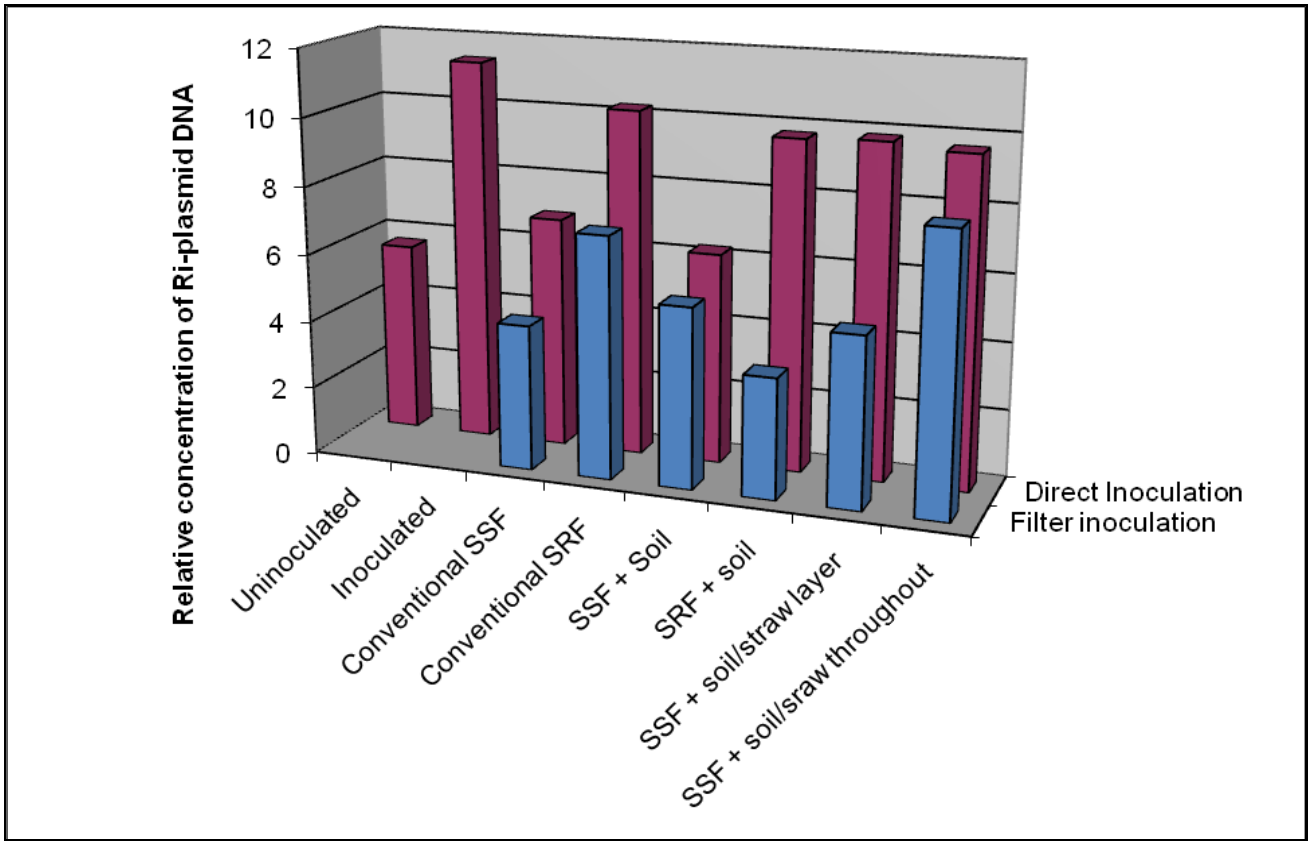
The root samples collected from symptomatic plants during the 26th June assessment were subjected to Rol-DNA real time PCR at CSL. All 3 of the samples from the trial were positive, whilst the sample from a separate tomato crop elsewhere at STC was negative. However, none of these 3 plants were showing symptoms at the end of the trial period.

The lack of root-mat development in this trial remains an enigma. Plants were inoculated with a culture of *Agrobacterium* stored in a frozen collection at CSL as it had proved impossible to isolate a fresh pathogenic strain in time for the inoculation. Although isolates from cucumber had previously been found to have retained virulence during storage, it is possible that virulence had declined in the isolate stored at CSL and used in the studies during 2007. A second line of inoculation using affected root and rockwool material supplied fresh from the affected nursery was also included in the 2007 trial, which also did not seem to aid symptom expression. However, we now also know that there was a marked decline in natural infection at the same nursery during

2007 and this may also suggest a weakening of the virulence or perhaps the build-up of suppressive or antagonistic organisms on the site.

Root samples collected at the end of the trial from plants in each plot were tested for the presence of the Ri-plasmid using TaqMan PCR. The results are shown graphically (Chart 2). Four root samples were collected from each row (plot) in the trial and, as in the 2006 trial the samples were collected from the 1st, 5th, 10th and 15th plant in each row. The chart shows the mean of the four values recorded/plot.

Chart 2. Representation of the relative concentrations of Ri-plasmid DNA in the root samples collected at the termination of the 2007 glasshouse trial.



Results of these tests in the previous (2006) trial had shown that a much higher concentration of Ri-plasmid DNA was found in the roots of the plants which had received a direct inoculation as well as via the filter compared to those plants which had received inoculum via the filter only (2006 chart shown in Appendix 3 for comparison). The data generated following sampling in the 2007 trial do not show this effect quite so clearly. Lower concentrations of DNA were seen in the roots of the plants which received inoculum via the filter only in all the treatments; however the differences in DNA concentrations detected are far less pronounced. Higher plasmid DNA concentrations were observed in the 'direct' inoculated plants in 2006, with typical values of

between 9 and 13[3], the values were more consistent across the treatments. One of the plants sampled (out of a total of 8) in the uninoculated control rows also tested positive for the presence of the Ri-plasmid. There is more variability in these values in the 2007 results with values between 6 and 11. In the filter inoculated plants values of between 0 and 3.5 were observed and these were increased in 2007 to 3.6 to 8.2. The reason for these variations is not clear. However it is important to note that the results of these analyses do show the plasmid to be present in all the roots of all the inoculated plants, despite the lack of symptom expression. There is evidently some factor which is preventing either the transformation process or symptom expression in these trials and if this could be determined it would potentially provide valuable information to formulate a control strategy to alleviate root-mat on commercial nurseries.

It is hoped that further work being carried out in 2008 using slow sand filters installed at the two UK commercial nurseries with known root-mat problems will provide the much needed information on the efficacy of slow sand filters on controlling root-mat in tomatoes.

[3] Nominal value calculated from PCR CT score.

Objective b: Investigation into the control or suppression of root-mat in tomatoes using existing microbial products

The results of the microbial product initial tests which were carried out on cucumber plants are shown in Table 3.

Table 3. Results of the small-scale experiments on Aviance cucumbers following application of microbial products.

Treatment / Plant	pRi PCR on 18.5.07	pRi PCR on 3.8.07	First cube symptoms	Slab Symptoms (at end)
Positive control 1	+	+	28.6.07	None
Positive control 2	+	+	-	
Positive control 3	-	+	-	None
Positive control 4	-	+	10.7.07	
Negative control 1	-	+	-	None
Negative control 2	-	+	-	
Negative control 3	-	+	-	None
Negative control 4	-	+	-	
Biomex 1	+	+	-	None
Biomex 2	+	+	-	
Garshield 1	+	+	24.7.07	Yes
Garshield 2	+	+	30.7.07	
GLD 1	+	+	22.6.07	Possible
GLD 2	+	+	-	
Seasol 1	+	+	-	Yes
Seasol 2	-	+	-	
PHC Compete 1	+	+	10.7.07	None
PHC Compete 2	+	+	-	
Gliomix 1	+	+	-	None
Gliomix 2	+	+	-	
FZB 1	+	+	-	Possible
FZB 2	+	+	24.7.07	
Stimagro 1	+	+	22.7.07	Yes
Stimagro 2	+	+	7.8.07	

At the end of the experiment plants treated with Gliomix and Biomex-SA (and negative control) were free from root-mat symptoms. All other treatments had some weak symptoms in the cubes and/or slabs by this time (Figures 4 & 5 overleaf).

The work is to be repeated on tomato during 2008.

Figure 4. Cucumber plants at the end of the trial following inoculation and treatments with Biomex SA.

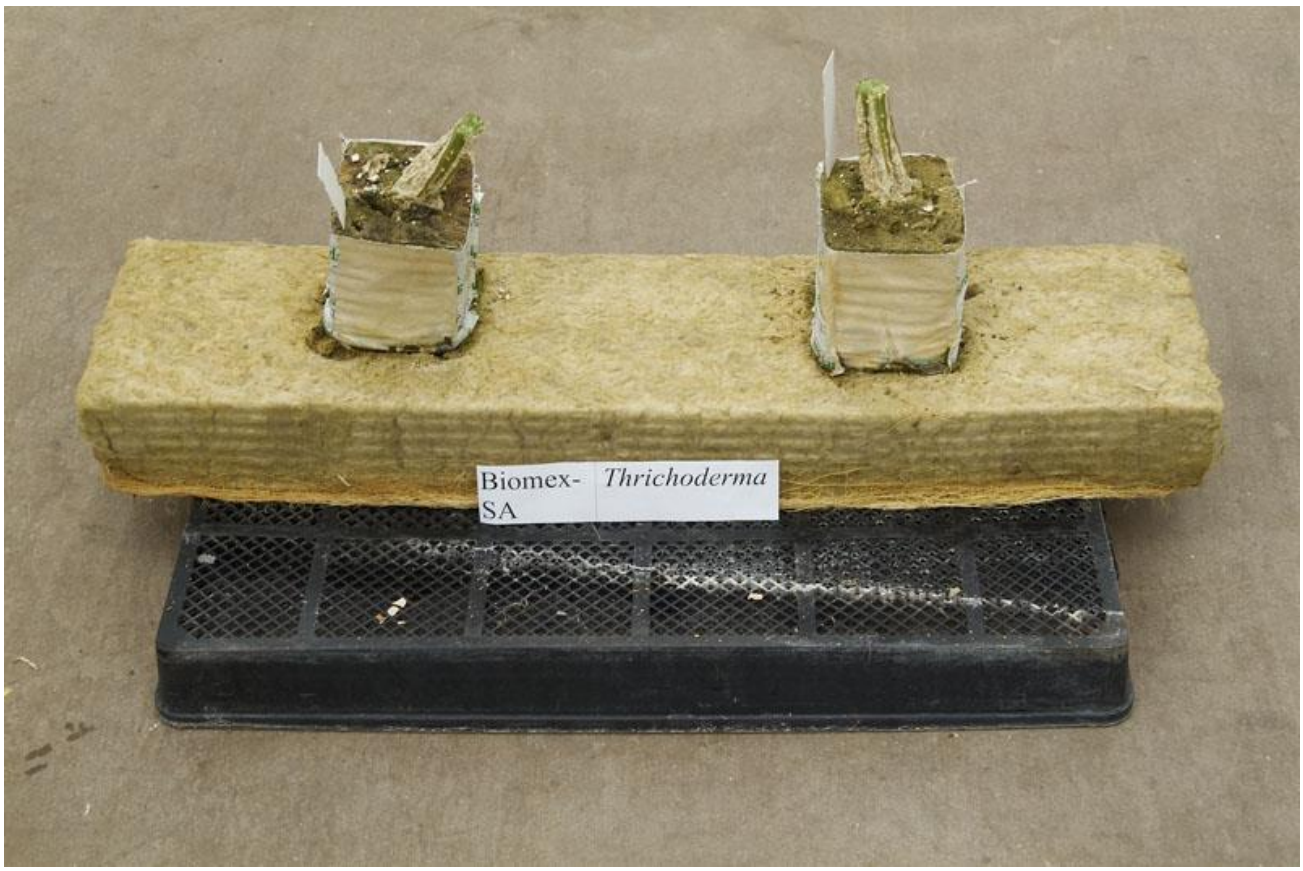


Figure 5. Weak root-mat symptom expression in cucumber plants treated with Garshield

Objectivec : Investigation into the potential of grafting onto alternative rootstocks e.g.

Aubergine as a means of suppressing or preventing root-mat in tomatoes.

The grafted plants integrated effectively into the commercial crop and observations of the crop were carried out over the season by the nursery personnel. No root mat symptoms developed in any of the grafted plants, though this cannot be considered a positive result as the incidence of root-mat in all crops at the nursery was much lower than had been seen in previous seasons. More importantly, it was also reported that the yield was dramatically reduced as a direct result of grafting onto an aubergine rootstock with an overall reduction of approximately 80% of that expected for the adjacent grafted tomato crop. This factor alone would make this control option unacceptable in a commercial environment, and therefore it is not proposed to take this control concept further.

Objective d: Investigation into the control or suppression of root-mat in tomato using cross-protection

Additional work on this objective is still on-going in a small glasshouse experiment being carried out at STC during the early part of 2008. It is too early to comment on results of this work at this time, and the results will be reported later.

Discussion

The work carried out in Year 2 of this project has again been frustrated by the lack of good symptom expression in the glasshouse study carried out at STC during 2007. In the STC trial it was particularly disappointing after early symptoms were observed in many of the plants for these to reduce over time and ultimately disappear in most of the plants (including the inoculated control plots). As a result of this it is not possible to draw firm conclusions regarding the disappearance of symptoms in the 'treated' plots or the efficacy of the applied treatments. The absence of symptom expression in the various trials conducted over the last two years is a real conundrum, particularly as the PCR tests on the samples from the trials indicated that *Agrobacterium* carrying the Ri-plasmid was present in the roots. Clearly some unknown factors are inhibiting symptom development. If this issue could be elucidated it could potentially pave the way towards developing commercially viable control options for both tomato and cucumber growers.

In an effort to try and investigate what these 'factors' might be additional studies and investigations will be undertaken during 2008. Firstly, the project team believe that it is important to further investigate the significance of infection timing on tomato plants. This is an aspect that has not been studied previously in tomatoes, but may prove important. Both glasshouse trials carried out at STC in 2006 and 2007 have been initiated (planted) in May of each year. Commercially, tomatoes are planted much earlier than this, usually in January for instance. We do not know when plants on commercial nurseries become infected with the *Agrobacterium* carrying the Ri-plasmid, however we know that symptoms are seen from April onwards in tomato (February onwards on cucumbers). Previous *in vitro* tests carried out at CSL on cucumbers as part of the studies sponsored by the HDC (PC 149) and Defra (HH2308SPC) have shown that symptom development usually occurs 6-8 weeks post inoculation. Extrapolation of this information suggests that infection of nurseries may be occurring during February and March. It is possible that some unforeseen factor e.g. increased temperature, or higher light levels may be impacting in a negative way on the ability of the plasmid to 'switch-on' the root proliferating gene in the tomato roots in our trials with later starting dates. Experiments at STC during 2008 will investigate the effects of inoculation on different age tomato plants planted earlier in the year. A range of isolates collected from two commercial nurseries where root-mat has been reported will be used in this study.

The reported natural decline of symptoms observed at the commercial nursery in southern England is also of considerable interest and may have potentially commercial significance. Once again it is unclear what factors may have led to this situation. Is this perhaps due to a natural loss of pathogenicity/virulence of the plasmid, or an increase in suppressive or antagonistic micro-flora? Have environmental factors such as light intensity (reportedly lower in 2007 than in previous years) had an effect on symptom development? It will be interesting to observe whether the trend continues during 2008, or whether symptoms return to previously reported levels.

The lack of consistent symptoms at the commercial nursery in 2007 resulted in an inability to draw firm conclusions regarding the potential benefits of grafting plants onto an aubergine root stock (that has not previously shown symptoms). However, the severe reduction in yield which was observed in this graft combination renders this approach non-viable economically. Therefore further work on grafting will not be pursued.

The studies carried out at CSL during 2007 investigating the potential benefits of commercially available microbial products have provided some useful information. Root-mat symptoms failed to develop in the cucumber plants which had received regular applications of Biomex SA, a *Trichoderma* based material or Gliomix, (*Gliocladium*), however, root-mat symptoms observed in the remaining treatments and the control plants were weak. These results are promising and a similar study on tomato plants, inoculated with a strain of rhizogenic *Agrobacterium* recently isolated from symptomatic plants in a commercial nursery will be conducted at CSL in 2008.

Information supplied by one of the Grower Co-ordinators on this project regarding the development of root-mat symptoms at another commercial nursery, this time in the north of England, during 2007 has provided us with an opportunity to gather further information and set up additional experiments on a root-mat infected tomato nursery. This may be particularly important if symptom expression is declining at the southern commercial nursery site, as we know that symptoms were quite severe in several crops at the new site during the same period. We are keen to make good use of this unexpected situation, gaining greater insight on this crop problem.

Conclusions

- A large-scale glasshouse trial on a hydroponically raised tomato and cucumber crop linked to 6 slow sand or slow rockwool filters was carried out at STC during 2007.

- Early root-mat like symptoms were observed in the crop predominantly on the tomato cv. Elegance plants. Symptom incidence and severity reduced over time in the crop, and only 5 plants were showing weak symptoms at the termination of the trial in November 2007.

- The absence of good symptom expression in the inoculated control plots means that firm conclusions regarding the possible efficacy and impact of the various filters on root-mat control in this study cannot be drawn.

- Root samples from the crop which were analysed using real-time PCR, showed the presence of the plasmid in the inoculated plots. Higher quantities of the plasmid were present in the majority of the plots which had been directly inoculated, whilst those plants which had only received inoculum via the filter showed some reduction in the level of plasmid DNA present.

- Further experiments were carried out at CSL to investigate the possible benefits of readily available microbiological products to control root mat. Root-mat symptoms were observed in the majority of blocks or slabs after 15 weeks. The plants which had received regular applications of either Biomex SA or Gliomix (along with the negative control plants) did not show any root mat symptoms at this time.

- The lack of symptom development in the various studies at CSL and STC continues to be of concern. Additional experiments to investigate the effect of timing of application and age of plants will be carried out in 2008 together with a small-scale experiment to investigate cross-protection and also to look for bacteriophage active against *A. radiobacter*.

- The report in 2007 of a further outbreak of root-mat at a commercial nursery in the north of England has provided the project team with the opportunity to install a second slow sand filter system (along with the initially chosen site in the south of England) in a crop which saw severe symptoms in 2006 and 2007. Small-scale microbial studies will also be carried out at one, or possibly both of these sites.

Technology Transfer

- Root mat update: Seminar to Wight Salads managers, Arreton Valley, Isle of Wight, 19th April 2007. (Tim O'Neill)
- A number of interested grower groups which have visited STC during 2007 were shown the tomato glasshouse trial.
- Derek Hargreaves visited the crop on several occasions during 2007 to provide technical input to the R&D programme.
- Several visits have been carried out to-date to the affected nursery in the north of England to discuss the problem and instigate a small scale study.

Acknowledgements

The financial support of the UK tomato industry, through the Tomato Growers Association and the Horticultural Development Council for this work is gratefully acknowledged. The granulate rockwool used in the filters and the 'know-how' was supplied free of charge courtesy of Sven Erik Lanng & Andy Lee of Grodan BV. The tomato seed for the trials was supplied by Wight Salads Ltd. Such industry support has been much appreciated. Finally, special thanks to Dr Phil Morley, Wight Salads and Derek Hargreaves, Consultant who, as joint project co-ordinators, have invested a considerable amount of their own time in support of this project. Thanks also to Andrew Clark and Dave Wilson at Plantraisers for undertaking the grafting work with aubergine and tomato. Also thanks to Enza for providing the aubergine seed.

Further Information

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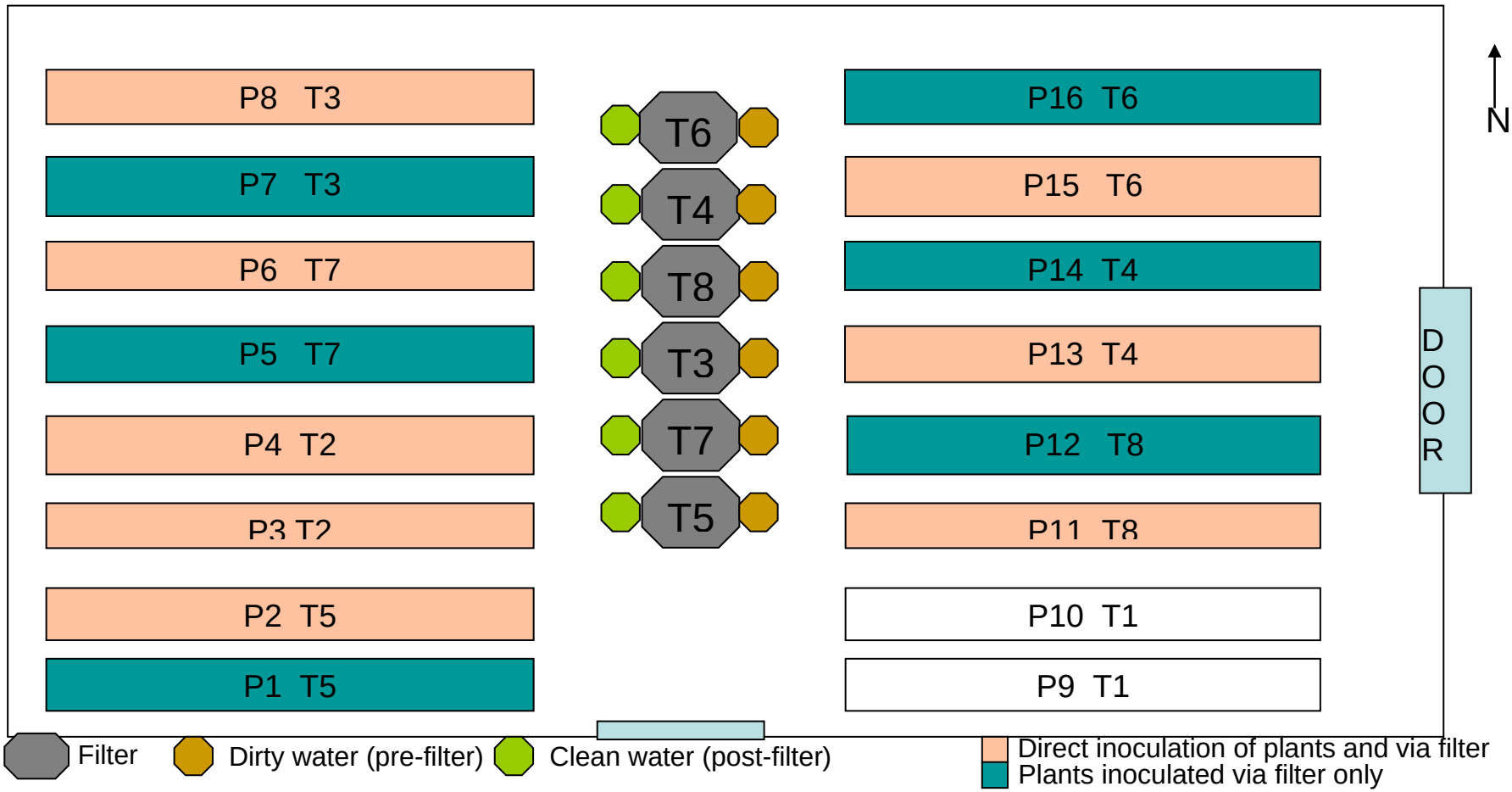
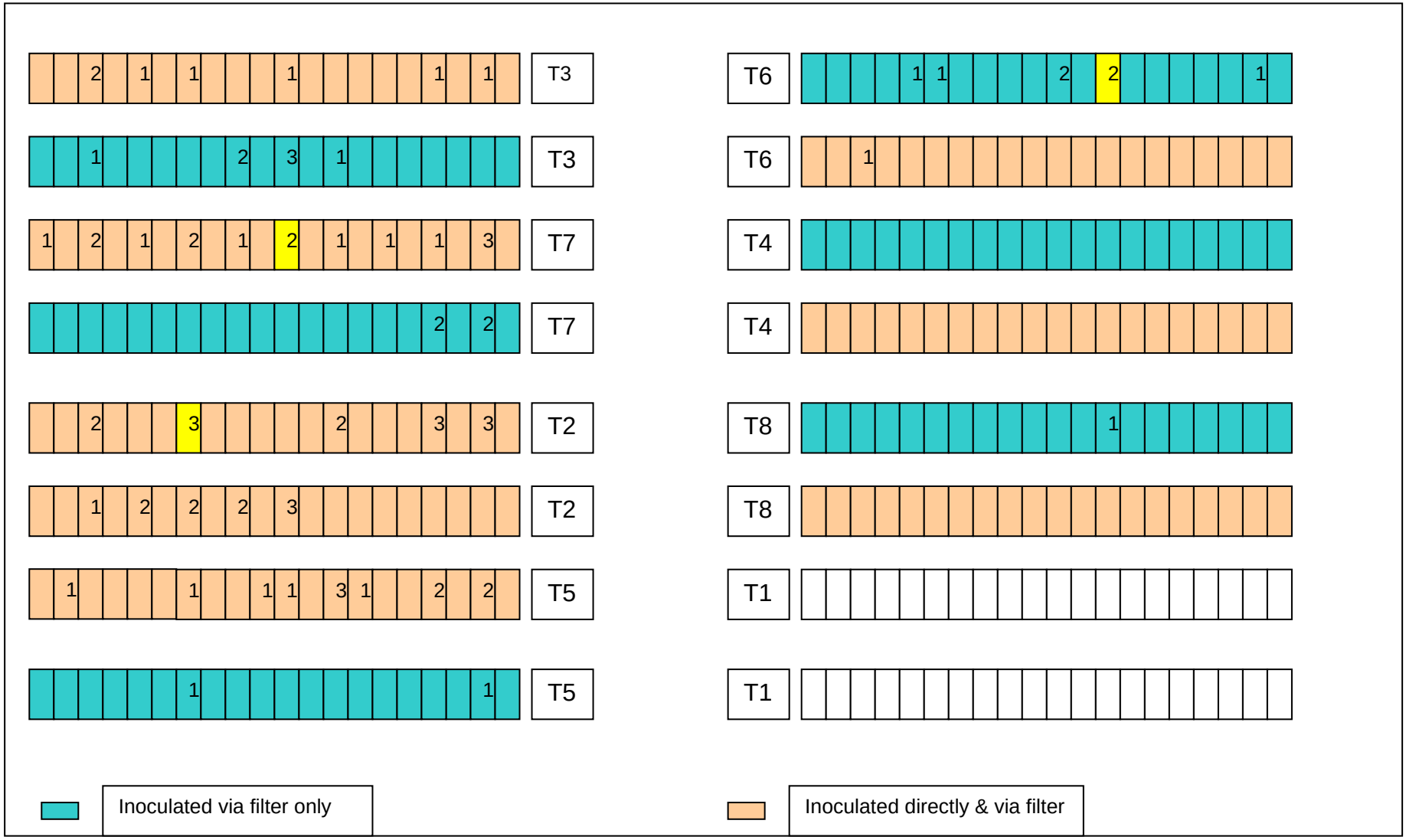
Appendix 1. Investigation into control of Root mat in Tomatoes
E301 2007 Trial Plan



Treatments **Tom cvs. Claree & Elegance** **cuc - Aviance**

T1 – Uninoculated control – RTW
T2 – Inoculated control – RTW
T3 – Inoculated + conventional SSF
T4 – Inoculated + slow rockwool filtration (SRF)
T5 – Inoculated + SSF with organic soil
T6 – Inoculated + SRF with organic soil sandwich
T7 – Inoculated + SSF with soil/straw sandwich
T8 – Inoculated + SSF with straw/soil mix throughout filter.

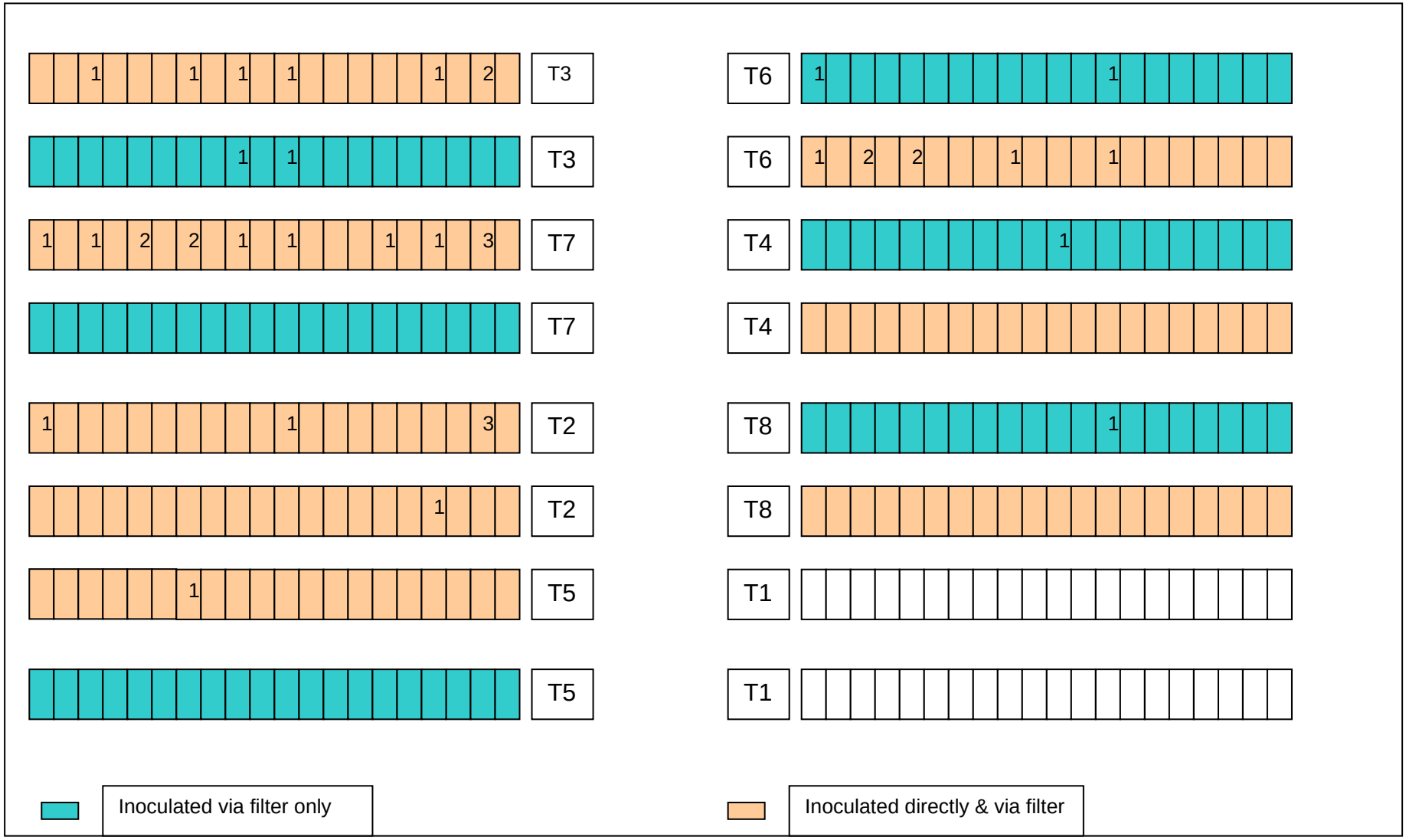
Appendix 2 Raw data from block root-mat assessment – 26.6.08



NB – Cucumber plants not included

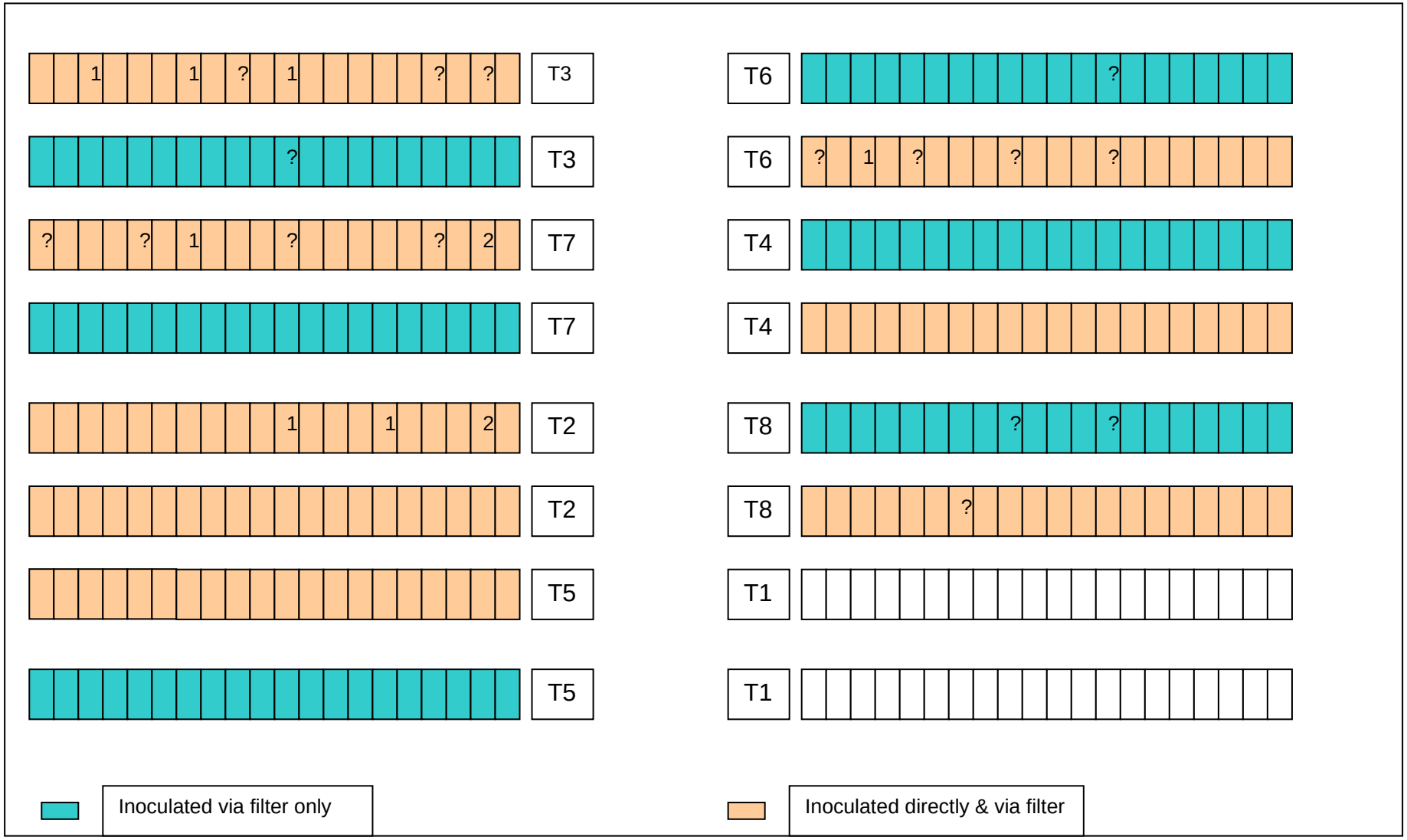
Highlighted boxes indicate plants where root samples were collected for PCR analysis.

Appendix 2 Raw data from block root-mat assessment – 7.8.07



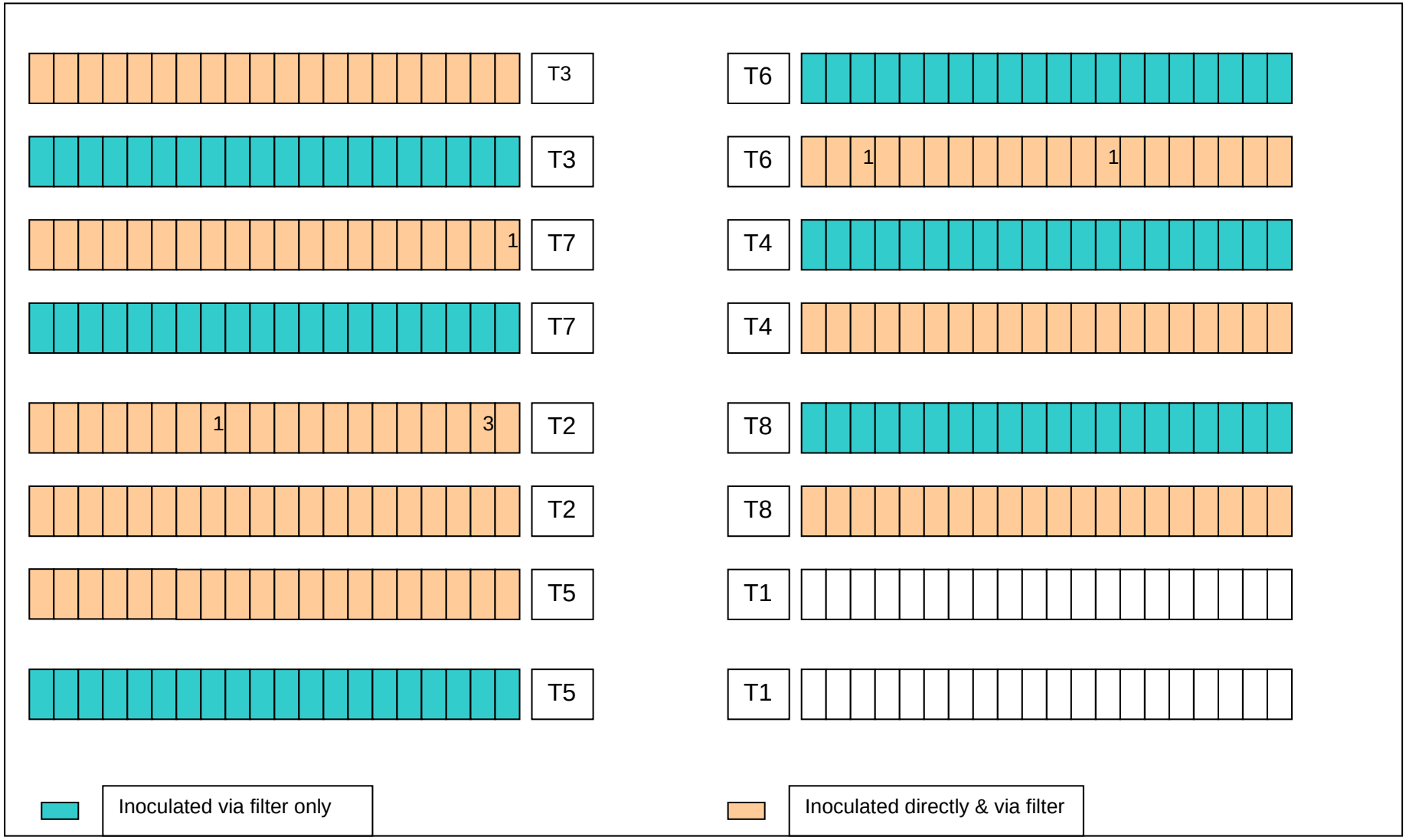
NB – Cucumber plants not included

Appendix 2 Raw data from block root-mat assessment – 30.8.07



NB – Cucumber plants not included

Appendix 2 Raw data from block root-mat assessment – 1.11.07



NB – Cucumber plants not included

Appendix 3

Chart 2. Results of PCR testing on root samples from the trial