Project Title:	Protected hydroponic tomato: investigating the potential for various novel non-chemical techniques for the suppression or control of root-mat disease.
Project Number:	PC 241
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Previous Reports:	Annual Reports, 2007 and 2008
Report:	Final Report, March 2009
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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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Date commenced:	April 2006
Completion date:	March 2009
Keywords:	Tomato, *Solanum lycopersicon Agrobacterium*, Ri-plasmid, slow sand-filtration, rockwool, hydroponic, root-mat, cross-protection, grafting, recirculation, mad roots, root proliferation, bio-control.

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The results and conclusions in this report are based on an investigation conducted over three years. 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.

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CONTENTS

Headlines

Previous work has been unsuccessful in controlling root-mat disease in tomato through chemical intervention. This project has investigated the potential of non-chemical techniques for suppression or control of root-mat in tomato. There is some scientific evidence that:

a) The greatest infection risk occurs around 4 weeks post-sowing
b) Increasing the biological activity/complexity may help suppress the disease, suggesting that a normal clean-up routine should be maintained between crops, avoiding high hygiene scenarios unless other pathogen outbreaks dictate decision.

Failure to achieve symptom expression in small-scale trails in the first two years of the project , together with a low incidence of root-mat on the commercial nurseries selected prevented a full validation of the biological suppression concept. Further studies would be required to confirm this alternative approach to root-mat control.

Background and expected deliverables

Root-mat was first reported in the UK in the 1970s on soil and straw bale grown cucumbers. It is caused by a small circular DNA element called an Ri-plasmid (Ri = root inducing) which is harboured by rhizosphere-associated bacteria, primarily from the genus *Agrobacterium*. 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 tended to reduce in recent years. This may perhaps be due to the reduction in the number of infected, but symptomless plants ex-propagation or perhaps due to a change in cropping frequency, thus reducing the impact of the disease on individual short-term crops.

The disease has now also appeared in tomato crops where it has been seen at a severe level in hydroponic crops on some nurseries in the UK over the last 4-5 years. 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.

Plate 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 suggestion that increasing the population of naturally-occurring microbial antagonists might suppress or prevent the development of root-mat commercially. This study was initiated as an alternative ‘biological’ strategy for root-mat control to investigate a number of alternative, non-chemical strategies to try and minimise or eliminate the risk of root-mat in hydroponic tomatoes.

Summary of the project and main conclusions

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.

However as additional questions have been raised over the duration of the study some extra objectives have been added:

e) To investigate the effect of inoculation timing on symptom development.
f) To monitor the development of root-mat in time and space in a commercial crop.
g) To conduct isolations for bacteriophage to *Agrobacterium*.

<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 a range of 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 a strain of *Agrobacterium* (supplied by CSL) containing the Ri-plasmid 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 and the study was terminated.

Year 2 (2007)

Following a project review meeting the above work was repeated but with significant modification. In 2007 two tomato cultivars – Claree and Elegance were used together with two cucumber plants, cv. Aviance per plot as 'indicator' plants. Previous experience at CSL had shown symptom expression, following artificial inoculation, to be stronger in cucumbers than tomatoes and hence these cucumber plants should act as an effective early indicator of root-mat in the trial. Artificial inoculations were carried out on the crop when the plants were relatively young (7 weeks after sowing) to try and aid infection. However, once again symptoms failed to develop strongly in either cucumber or tomato, despite early signs of infection in the crop.

The lack of symptom expression in these trials 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 e.g. the host plant and abiotic factors.

During the same period it was reported that root-mat had occurred at a tomato nursery in the north of England, and that it was quite severe in 2007 so the absence of root-mat symptoms was clearly unlikely to be a result of local environmental factors. Work in 2008 therefore focused on commercial nurseries at both the known affected sites to help ensure that some useful data and information could be gathered.

Year 3 (2008)

Experimental slow sand filtration units were installed at two commercial tomato nurseries, one in the north of England and the other in the south of England. The systems functioned well; however, yet again, very low levels of root-mat were seen at both sites during 2008 and this made drawing positive conclusions from the work very difficult.

<u>Objective b</u>: 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. As with the glasshouse trial at STC, very poor symptom expression was seen and few conclusions could be drawn except that in molecular tests eight weeks post-inoculation rhizogenic *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.

Year 2 (2007)

Following the disappointing results seen in Yr 1, the work was repeated using young cucumber plants as this crop was deemed to be more likely to show some symptom expression. The following list of microbial products was used in 2007.

Details of Bio-control products evaluated in 2007

Treatment	Supplier	Active ingredient or organism	Rate of application (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 developed on the inoculated plants in all treatments except those treated with either Gliomix or Biomex SA. The negative control plants also remained free of root-mat symptoms.

Year 3 (2008)

Experiments similar to those described above but focusing on Gliomix and Biomex SA were repeated in tomatoes at CSL using an isolate of *Agrobacterium* recovered from symptomatic plants in a commercial nursery in November 2007. The presence of rhizogenic *Agrobacterium* was demonstrated, using molecular tests, in all plants inoculated with the root mat pathogen, even before symptoms were evident and regardless of whether plants were treated with microbial products. Some very slight unusual root growth was observed in the inoculated untreated plants, whilst no similar symptoms were observed in the treated plants, however symptom expression was weak at best making it difficult to draw firm conclusions.

A range of products were also tested on a nursery. Two experiments were done in commercial crops of tomato grown on rockwool slabs. Both experiments were located in glasshouses with a history of root-mat disease.

In the first experiment (an unreplicated observation study), the microbial products Gliomix (*Gliocladium catenulatum*), Biomex (*Trichoderma* sp.) and Trianum P (*Trichoderma harzianum* strain T-22) were applied as drench treatments to cubes, twice in propagation (December 2007) and once post-planting (February 2008). Root-mat first appeared in the crop in April and increased to affect 11% of untreated plants by mid-June. Gliomix and Biomex did not reduce the disease. Trianum P appeared to reduce the disease and treated rows had just 3.7% plants affected by September. However, no firm conclusions can be drawn due to the large variation in disease incidence between the duplicate untreated areas (17.0% and 4.2% in September).

In the second experiment (a fully randomised replicated trial), the microbial products Ecoguard GN (*Bacillus licheniformis*) and Rhizopro (*Bacillus subtilis* var. *amyloliquifaciens*) were applied as drench treatments to cubes on four occasions between 19 February and 10 April 2008. Root-mat first appeared in mid-April and increased to affect 13% of untreated plants by November. Neither product significantly reduced the incidence or severity of root-mat at any of six assessments done monthly.

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 noted that the incidence of root-mat on rockwool crops across the same nursery was also dramatically reduced compared to previous years. This factor is intriguing and perhaps suggests either a build up of naturally suppressive organisms or a natural weakening of the plasmid's virulence. Whatever the cause, it unfortunately meant that firm conclusions could not be drawn regarding the effectiveness of the aubergine rootstock in suppressing root-mat. However, it was observed that the graft combination impacted severely on yield, reducing it by approximately 80% - this alone would result in this potential solution being unacceptable unless a significantly more vigorous aubergine or other root-stock could be sourced and used instead.

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 a different Ri-plasmid than at nursery B. This suggests a correlation (in this instance) between Ri plasmid type and symptom severity. Work was carried out to investigate whether inoculation with *Agrobacterium* containing the less virulent plasmid would provide any protection when the plants were challenged at a later date with the more virulent plasmid type. Unfortunately, a lack of symptom throughout all the treatments again meant that no firm conclusions could be drawn during the experiment.

Year 3 (2008)

A small, though fully replicated and randomised trial was carried out at STC. Tomato cv Claree were grown on rockwool slabs. Two isolates of *A. rhizogenes* + pRi were inoculated onto plants either individually or following 'inoculation' with the less virulent strain of root-mat identified during the 1st year of this study. Low levels of symptom expression was seen in a few plots, however no clear patterns of infection were seen and it was not possible to confirm or discount the 'cross-protection' hypothesis.

Objective e: Effect of inoculation timing on symptom development

Year 3 (2008)

A small scale but fully replicated tomato trial was carried out at STC to investigate the effects of a range of different inoculation timings on symptom development. Two different isolates of *A. rhizogenes* + ri-plasmid were used and young Claree tomato seedlings were inoculated at 0, 4, 8 and 12 weeks post sowing. In this trial root-mat did develop and consistent symptom expression was observed in the plants, particularly in those inoculated at 4 weeks post-sowing. There was further symptom development in other plants inoculated earlier and later in the trial but infection levels were much reduced in comparison. This information suggests that there may be a very narrow window for optimum infection. This 'window' appears to be very early in plants; and yet symptoms don't develop until 2-3 months later. Therefore any hygiene or alternative crop protection measures should be implemented from sowing onwards e.g. during propagation.

Objective f: Monitoring development of root-mat in time & position

The development of root-mat was monitored in 22 rows of tomato cv. Jack Hawkins grown on rockwool slabs untreated with microbial amendments. Root-mat was first observed on 15 April (3% of slabs affected) and increased to affect 12% of slabs by 21 June. Disease occurrence did

not increase any further during the remainder of the season. Symptom severity continued to increase as the season progressed.

The occurrence of typical symptoms across the crop of slabs containing plants affected by root-mat was uneven. There was a large difference in the occurrence and severity of symptoms between two areas of the monitored crop (separated by 33 rows) where there were 17% and 4% of slabs affected in November. There was a trend of increasing occurrence across the house from the central concrete pathway to the sidewall. Although most affected slabs were bounded by unaffected slabs, there were a few groups of up to six adjacent slabs with symptomatic plants along a row. Grouping of affected slabs along a row was considerably more common than grouping across a row. This may reflect disease spread along a row from slab to slab, or possibly latent (symptomless) infection in a specific tray of plants at planting.

Objective g: Isolation of bacteriophage to *Agrobacterium*

Attempts to isolate bacteriophage were made from rockwool and root-material as well as water samples taken from nurseries where symptoms of root-mat had been observed to decline in symptom severity. Unfortunately no phage pathogenic to the root-mat carrying organism was detected. This may be an area that warrants further work in the future.

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, reducing 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 during seasons of severe root-mat occurrence were in the region of £0.75M/annum though the potential for greater loss is considerable now that the problem is also known to occur on other tomato nurseries in the UK.

Unfortunately progress in this project has been hampered severely by a marked decline in the severity and occurrence of root-mat in both commercial and experimental crops particularly during 2007 & 2008. We may be experiencing a general decline in root-mat occurrence in tomatoes, as has been seen previously in cucumbers. Alternatively, the cool, wet summers experienced in the

last 2 years may have adversely affected the pathogen, though past experience suggests that we are likely to see the problem again in further seasons.

Action points for growers

- Liaise with propagators regarding hygiene measures and possible applications of biological control products pre-delivery to nursery.

- Consider the use of biological products during the 1st two months after the crop is planted.

- Maintain a normal clean-up routine between crops, avoid high hygiene scenarios unless other pathogen outbreaks dictate decision.

- Monitor crops and feed information back to members of the project team if root-mat problems increase on your nursery.

Project Co-ordinator Comments

When dealing with this disease in the production nursery it would appear to be best to do no more than a normal clean-up. Trying to do more than this may increase the level of the problem rather than reduce it. However, clean-up action in propagation to reduce and remove early infection does seem to be more effective in reducing the level of infection in the mature crop. This action in the propagation area should include the trays used for delivery as these may carry infection back from infected nurseries into the propagation nurseries.

Derek Hargreaves

Experience with this problem suggests that 'dirtier is cleaner' in any attempt to get rid of this problem we need to understand more about the microbial competition. Areas diligently cleaned with several biocides seem no more cleaned up of the plasmid than moderately cleaned areas. Growing in soil appears to be the best option resulting reduction of symptoms.

It also appears that plant stress plays an important role in symptom expression with increased stress resulting in more severe symptoms.

Dr Phil Morley

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 since around 2000, 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.

Plate 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 (Plate 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 is not economically viable and a single, long-term crop is grown each season. The ultimate aim of the project was 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 and 2008. Separate elements of the work in 2007 were 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.

Also following discussion at project review meetings, additional work was undertaken in 2008 on three further objectives:

e) To investigate the effect of inoculation timing on symptom development.
f) To monitor the development of root-mat in time and space in a commercial tomato crop.
g) Isolation for bacteriophage pathogenic to *Agrobacterium*.

Materials and methods

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

In 2007 and 2008 the project team helped two commercial tomato nurseries with severe root-mat problems to install slow sand filtration systems on their own sites. At Nursery A (in southern England) a filter (Plate 3) was set up which provided filtered water to one row (84 blocks) in a glasshouse.

Plate 3. Slow sand filter arrangement at Nursery A

The SSF recirculation was applied from planting (cv Jack Hawkins). The rows either side, which were on run-to-waste irrigation, were used as the untreated control. All of these rows were also treated with Trianum P by the grower as another protectant measure to alleviate problems with root-mat on site. As both the treated (filtered) and untreated (unfiltered) water each received the same Trianum treatment it was possible to continue with the SSF investigation at this site.

Plants were examined monthly from March to November and the incidence and severity of root-mat symptoms was recorded, as described in previous annual reports.

At Nursery B (northern England) two smaller filters which were the same as those used during the STC experiments were installed (Plate 4). These were used to irrigate a single row (96 plants) cv Dirk within an area of the glasshouse where root-mat had been problematic in 2007.

Plate 4. Slow sand filter at Nursery B

The plants in the 'filter' row and the 'untreated' rows on either side were monitored for the development of root-mat symptoms every two weeks. At the end of the season 75 samples were collected (25/row) for testing using the molecular *rol*-PCR test developed at CSL.

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

In 2008 tomato seed, cv Claree, were sown in rockwool cubes. Plants were treated weekly with either Gliomix® (Fargro Ltd, W. Sussex) or Biomex SA (Plant Solutions Ltd, Surrey). For each amendment, three treatments were established: weekly inoculation commencing on the same day as seed were sown, weekly inoculation commencing two weeks after sowing and no inoculation. The products were used at the same rate as in the 2007 experiment. Each plant was treated with 25 ml of Gliomix, Biomex or water as a negative control.

Seedlings were inoculated 18 days after sowing by pouring 20 ml suspension of *Agrobacterium* strain P6994 (isolated from root-mat symptoms at a commercial tomato nursery in 2007) at a concentration of approximately 10^7 cfu/ml in phosphate buffered saline. For each treatment, uninoculated negative control plants were also included.

Seven weeks after sowing, rockwool cubes containing seedlings were transferred to rockwool slabs, each slab accommodating two plants. Irrigation was by automatic drip-feeding and plants were kept at 20ºC supplemented with 12 h artificial light per day. Plants were monitored daily for the appearance of root-mat symptoms.

A further set of experiments looking at the effects of microbial products was carried out at Nursery A during 2008.

Site and crop details

An observation study and a replicated experiment were done in commercial crops. The observation study was located in a crop of cv. Jack Hawkins (ungrafted) and the replicated experiment in a crop of cv. Temptation (ungrafted) both in southern England. Plants were supplied by Plant Raisers Ltd and were grown on new Cultilene rockwool slabs with three cubes/slab and two plants/cube.

Both experiments were located in glasshouses where, in the previous year, crops were badly affected by root-mat.

***Observation study*:**

1. Untreated
2. Gliomix (*Gliocladium catenulatum*) at 2 g/L
3. Biomex SA (a mixture of *Trichoderma harzianum, Trichoderma koningii, Trichoderma polysporum* and *Trichoderma viride*) at 1 ml/L
4. Trianum P (*Trichoderma harzianum* strain T-22) at 0.06 g/L
5. Untreated

Treatments were applied twice in propagation (6 and 13 December 2007) at a volume of 250 mL/cube and once post-planting (16, 19 and 20 February 2008 for Gliomix, Biomex and Trianum P respectively) at 200 mL/cube.

Gliomix is marketed by Fargro (www.fargro.co.uk) as a biological growth promoter. Biomex is marketed by Plant Solutions Ltd (www.plantsolutionsltd.com) as a soil applied growth stimulant. Trianum P is marketed from the Netherlands by Koppert BV (www.koppert.com) to enhance the resistance of plants to stress and diseases.

Replicated experiment

1. Untreated
2. Ecoguard GN (*Bacillus licheniformis* SB3086) at 2 L in 400 L water
3. Rhizopro (*Bacillus subtilis* var. *amyloliquifaciens* FZB24) at 80 g in 400 L water

The two biological preparations were recommended by Novozymes as suitable for suppression of root-mat. Both products are marketed in the USA. EcoGuard is marketed as a biofungicide and Rhizopro (under the name Taegro) is marketed to increase plant strength, enhance growth and suppress certain diseases. Neither product is currently available in the UK.

Treatments were applied four times, on 19 February, 5 March, 18 March and 10 April 2008, before the appearance of any root-mat symptoms in the crop. Treatments were applied by hand as a drench (100 mL/cube) over the cube surface.

Observation study

Each treatment was applied to 11 adjacent rows, a total of 924 cubes. The three treatments were located adjacent to each other. Untreated areas of 11 rows either side of the block of three treatments were used as duplicate untreated controls. For statistical examination, the observation study was treated as a non-randomised block trial with 11 replicates of five treatments. We made the assumption that the position of replicates does not influence the results. Disease incidence data was examined by regression analysis and disease severity data by ANOVA.

Replicated experiment

The experiment was a randomised block design with seven replicates of three treatments. Each plot consisted of 12 adjacent slabs (24 cubes) in a row, abutting onto the central pathway. The plants were examined as described above.

Assessments

Cubes were assessed for root-mat at approximately monthly intervals from mid-April, using the following scale:

0 - no symptoms
1 - occasional upright roots on cube (possible root-mat)
2 - cube surface largely covered by upright roots (definite root-mat)
3 - cube swollen out of shape due to excessive root growth
4 - collapse of root-mat affected roots
5 - death of plant (that has previously shown root-mat symptoms)

In the replicated experiment, at the end of cropping, slab wrappers were removed and the slab surface and sides were examined for root-mat symptoms (i.e. roots excessive in number). The top of the slab was also broken open to check for thickened corky roots. Disease severity in the slab was assessed on the following scale:

0 - no symptoms
1 - slight excess in numbers of roots in slab (possible root-mat)
2 - definite excess in numbers roots in slab (definite root-mat)
3 - thickened corky roots on slab

Results were examined as disease incidence (proportion of cubes categorised as index 2 or above) and disease severity (mean disease index).

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

A small-scale experiment to further investigate the potential for cross-protection was conducted at STC during 2008 using a range of root-mat isolates, some of which have been collected from additional affected nurseries.

Experiment design

Plants (cv Claree) were propagated and used in a fully randomised block designed trial. Six treatments were replicated in 4 plots with each plot comprising 4 plants (Appendix 4 Trial Plan).
All plants were grown in new rockwool blocks and slabs on a run-to-waste system.

Treatments

1. Uninoculated control
2. Inoculated with 'weak' strain (P5133) only
3. Inoculated with 'virulent' strain (P6994) only
4. Inoculated with 'virulent' strain (P6399) only
5. Inoculated with 'weak' (P5133) and 'virulent' (P6994)
6. Inoculated with 'weak' (P5133) and 'virulent' (P6399)

NB 'Weak' and 'virulent' designations determined by the severity of the symptoms seen in the crop at the time of isolate collection. P5133and P6399 were collected in 2006 from cv Claree, whilst P6994 was collected in November 2007 from cv Elegance.

The young plants destined for T2, T5 and T6 were inoculated with a turbid (approx 10^7 cells/ml) suspension of the *A. rhizogenes* + pRi isolate P5133 whilst still in propagation (approx 5 weeks post-sowing). Aliquots of 10mL/plant were applied. The 'virulent' strains were applied in the same way 2 weeks later.

Assessments

The plants were assessed on a fortnightly basis. The 0-3 symptom severity scoring system shown below was used to quantify the root-mat symptoms observed.

0 - No root-mat symptoms seen
1 - Few suspect, upright roots seen, no block swelling
2 - Block surface largely covered by upright roots (definite root-mat)
3 - Good root proliferation, block swollen out of shape due to excessive root growth

Additional experiments carried out during 2008

Objective e: Inoculum timing experiment

Following discussion at a project review meeting it was agreed that an additional experiment to determine whether timing of infection with the plasmid carrying bacteria was important in terms of epidemiology of the disease would be incorporated into the final year of the project. Root-mat symptoms are usually observed for the 1st time in commercial tomato crops in or around April. We decided to experiment with inoculating plants with 'virulent' strains from sowing onwards to see if there was a more susceptible period in the plant's development which would then result in a higher level of root-mat infection occurring.

A small replicated trial was carried out at STC. Tomatoes cv. Claree were sown and then inoculated with either isolate P6399 isolated from Claree in 2006 or with P6994 isolated from cv Elegance in 2007 (see trial plan Appendix 5). Batches of 16 plants were inoculated at 0 weeks (in the week they were sown), 4 wks, 8wks and 12 wks post-sowing with turbid (ca 10^6 cells/ml) suspensions of *Agrobacterium* carrying the respective Ri-plasmid strain. The plants were monitored and assessed every 2-3 weeks during the growing season.

Objective f: Monitoring development of root-mat in space and time

Observations at Nursery A suggest that root-mat occurs more commonly on slabs adjacent to the central concrete pathway than in the centre rows of a glasshouse. The objective of this study was to map the development of root-mat symptoms in space and time in two areas of a tomato crop untreated with microbial amendments, on the basis that this might provide valuable information in relation to disease epidemiology.

Materials and methods

The position of slabs with one or more plants in the slabs affected by root-mat was recorded in 22 rows (2 areas each of 11 adjacent rows). No biological amendments or fungicides were applied to the slabs. The overall proportion of slabs containing one or more plant affected by root-mat was determined on five occasions between 15 April and 3 November 2008.

Data were also examined to determine if there was any evidence for a greater occurrence of affected plants in a particular area of row by comparing the incidence of slabs containing an affected plant in 10 adjacent slabs close to the concrete pathway (slabs 1-10), 10 in the centre of the row (slabs 15-24), and 10 close to the outside glass wall (slabs 32-41).

Additionally, the occurrence of groups of adjacent slabs along rows and across rows containing one or more affected plant was determined for the first (21 May) and last (3 November) full assessments.

<u>Objective g</u>: Isolation for Phage pathogenic to *Agrobacterium*

Attempts to isolate bacteriophage were made from rockwool and root-material as well as water samples taken from nurseries where symptoms of root-mat had been observed. Rockwool and root-material (approximately 1 g each) was mixed with 10 ml sterile water by vortexing. A cocktail of five rhizogenic *Agrobacterium* strains recently isolated from nurseries in the UK was grown to a density of approximately 10^6 cfu/ml in 10 ml liquid medium, to which was then added 1 ml water sample or rockwool/root extract prepared as described above. Cultures were grown overnight with shaking at 25ºC before treating with 200 µl chloroform to kill bacterial cells. The culture was then centrifuged to remove cell debris and passed through a 0.2 µm filter membrane. Aliquots of the resulting supernatant were then tested for the presence of bacteriophage lytic on *Agrobacterium* as follows: 10 ml volumes molten agar medium (containing 0.6 % agar, w/v) were inoculated with 100 µl broth culture of one of the five rhizogenic *Agrobacterium* strains used in the original enrichment cocktail. Inoculated medium was mixed with 100 µl enriched supernatant which was then poured onto the surface of solid culture medium. After all media had solidified, cultures were incubated for up to 48 h at 25ºC and observed for the presence of cleared plaques indicative of phage lysis. Various media were used in attempts to isolate phage, as detailed below. In all cases, liquid culture media were the same as the solid media with the omission of agar.

NBSYE Medium: [0.8% nutrient broth (Oxoid), 0.5% sucrose, 0.25% yeast extract (Oxoid), 100 mM NaCl, 1 mM $MgCl_2$]

LB medium: [1% tryptone, 0.5% yeast extract, 0.5% NaCl pH 7.5]

LB with Mg supplement: [1% tryptone, 0.5% yeast extract, 0.5% NaCl, 1 mM $MgCl_2$ pH 7.5]

Where lacy growth, which may indicate phage-mediated lysis, was observed, a portion of this growth was removed and transferred to a sterile 1.5 ml micro centrifuge tube containing 1 ml liquid medium. This was incubated at 25ºC overnight before being assayed for the presence of plaque-forming *Agrobacterium* using the agar overlay method described above.

Detection and isolation of rhizogenic *Agrobacterium* from glasshouse samples

Detection of rhizogenic *Agrobacterium* in plant, rockwool and water samples was done by real-time PCR with primers specific to the rhizogenic plasmid following enrichment in Brisbane and Kerr's 1A broth (Brisbane and Kerr, 1983). For water samples, 100µl water was placed in 10 ml 1A medium. For plant and rockwool samples, approximately 0.5 g material was placed in 10 ml 1A broth. Inoculated medium was incubated with shaking at 28ºC for 72 h, after which time 100µl was removed and boiled for 6 min. A 2 µl aliquot of boiled enrichment was used as template for real-time PCR using primers rol-F (5'-ggc gat aaa acc ttc cag atc a-3'), rol-R (5'-gtc cgt gct cac aac att gc-3') and the probe rol-P (5'FAM-cgc acc gcc gcg tgg aa-T3'TAMRA) as described by Weller and Stead (2002).

For isolation of bacteria, approximately 100-500 mg material was vortexed with 1 ml phosphate buffered saline, and 100 µl aliquots spread onto Schroth's medium (Schroth *et al*., 1965). Plates were incubated at 25-28ºC and colonies typical of *Agrobacterium* were streaked onto fresh medium and a small aliquot boiled in water to be screened using the real-time PCR method described above.

Results

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

Slow-sand filters at commercial nurseries

Both slow sand filters, which were installed at the commercial nurseries with a history of root-mat, functioned well in 2008. Unfortunately for this component of the investigation, neither nursery experienced a very high incidence or severity of root-mat infected plants during 2008. At the southern nursery the plants in the 'filter' treated row were assessed monthly for the incidence and severity of root-mat and compared to the rows on either side which were on a conventional run-to-waste system, although plants in all these rows had been treated with Trianum P.

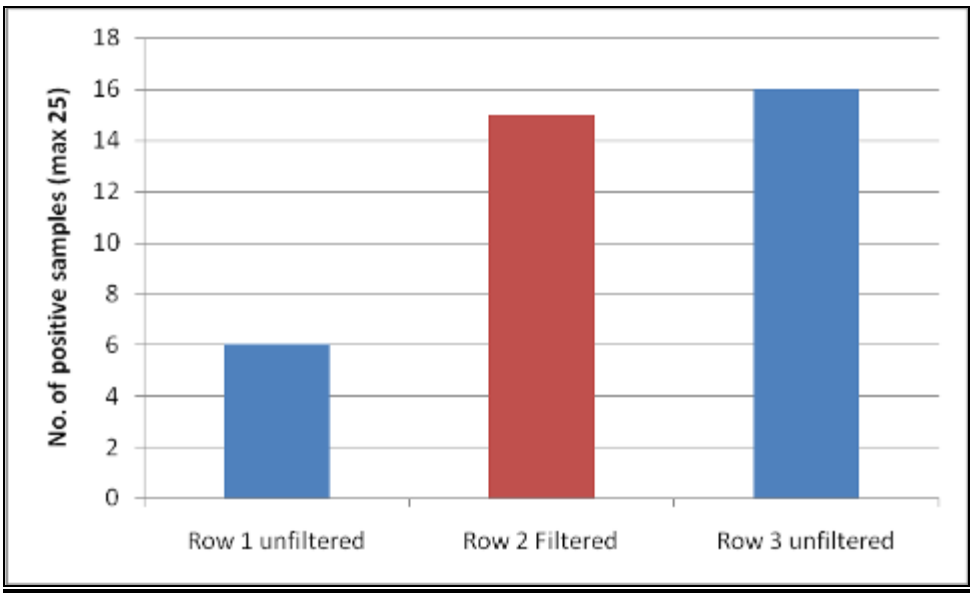
Root-mat symptoms (index 2 or greater) were first observed in a few cubes (2% of slabs) in the run-to-waste rows from May onwards, with a total of 3 slabs in each of these rows by the end of November. One slab with definite root-mat was observed in the SSF-treated plants from July with no further developments later in the season. Due to the very low incidence of symptoms in both treated and untreated rows, no conclusions can be drawn as to the effect of a slow sand filter on occurrence of tomato root-mat.

At Nursery B the situation was very similar. No definite symptoms were seen on the plants in any of the rows under observation, although unusual root development was seen on random plants throughout the season, the symptoms had often disappeared by the next assessment. The final assessment was carried out on the 29th October 2008. Root core samples were collected from 75 plants (25/row) and sent to CSL for testing using real-time PCR. Despite the fact that none of the plants tested were showing symptoms 53% of the samples tested positive for the Ri-plasmid (Figure 1). Similar numbers of positive samples were collected across the rows and this suggests that either the filter was unsuccessful in removing the root-mat plasmid from the irrigation water, or that the plants were infected prior to the filter being installed.

The scenario where plants are carrying the plasmid but are not expressing root-mat symptoms has been seen previously in trial crops and in samples collected from other commercial crops of tomatoes and cucumbers. It suggests that contrary to original thinking, the plasmid can be present in the root DNA but for some reason fails to 'turn-on' the genes to initiate root proliferation in the plant. There are several possible reasons for this. Firstly, timing of infection with the plasmid may be very important as shown in our timing inoculation experiments carried out at STC (and the earlier trials in cucumber), secondly the plasmid may be losing its virulence over time – and this may tie in with the disappearance of root-mat in cucumbers during the earlier history of the infection in the UK. Another possible reason is that the genes

inserted into the plant DNA may need triggers to allow them to be expressed e.g. particular light or temperature levels etc. Weather conditions in 2007 and 2008 were below optimum and this may be linked to the reduction in symptoms observed across the UK. Another possible factor could involve direct competition from other bacteria inhibiting infection or symptom expression.

Figure 1. The number of samples found to be positive for the Ri-plasmid (25 taken/row)



Whatever the cause for the lack of symptom development, it has resulted in an incomplete evaluation of the slow sand filters as a potential means of control for root-mat within the project. This has been very disappointing, however it may be possible to leave the filter installed at Nursery A so that it is active in subsequent years when root-mat may be more prevalent and this would allow the system to be evaluated further.

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

Ri-plasmid was detected by PCR in all tomato plants inoculated with *Agrobacterium* strain P6994 seven weeks post inoculation, regardless of whether plants had been treated with either Biomex or Gliomix during small-scale trials carried out on tomato plants in 2008/9. Severe symptoms were not observed on experimental plants, although early signs of root mat symptoms, visible as root growth on the surface of cubes, was apparent on those plants inoculated with rhizogenic *Agrobacterium* but not treated with microbial products.

Results of microbial product evaluation studies carried out at Nursery A

Suspect root-mat symptoms were first observed in early April around three months after planting. By 15 April 2008, definite root-mat (index 2) was present in both crops.

Observation study

By mid-May root-mat was evident in all treatments, at levels ranging from 3% to 13% of plants. There appeared to be a gradient of disease incidence across the experimental area (Table 1). Disease incidence increased slightly in all treatments during June, and then no further for the remainder of the season. Disease severity tended to increase between May and June and between July and September (Table 2). At the end of cropping in November, a total of 21 plants out of 237 (9%) with root-mat symptoms had died.

Although there were significant differences between treatments in both root-mat incidence and severity at the June, July and September assessments, no firm conclusions can be drawn on the efficacy of microbial amendments, due to the contrasting disease levels in the two untreated control areas (T1 and T5). The results may have been due to a gradient of disease from high in treatment 1 to low in treatment 5 across the experimental area of the glasshouse. Gliomix and Biomex had no effect on the disease. Trianum P may have reduced root-mat; the incidence of the disease was least in this treatment at all assessments.

Table 1: Effect of three microbial amendments on the occurrence of root-mat in tomato grown in rockwool slabs – observation study, 2008

Treatment	Mean % cubes affected (index 2 or greater)				
	15 April[a]	21 May	21 June	28 July	2 Sep
1. Untreated	3.6 (1.3)	12.9 (3.2)	17.8 (3.9)	17.2 (3.8)	17.0 (3.7)
2. Gliomix	9.2 (2.0)	9.3 (2.8)	15.3 (3.7)	15.9 (3.7)	15.1 (3.5)
3. Biomex	5.7 (1.6)	7.3 (2.5)	12.2 (3.3)	11.8 (3.3)	12.4 (3.2)
4. Trianum P	0.0	3.2 (1.8)	4.1 (2.1)	3.7 (2.0)	3.7 (1.9)
5. Untreated	1.4 (0.8)	4.7 (2.0)	5.1 (2.2)	4.2 (2.1)	4.2 (2.0)
Significance	0.001	NS	0.009	0.004	0.003

[a] 4 rows/treatment assessed; NS – no significant differences; ( ) – standard error.

Table 2: Effect of three microbial amendments on the severity of root-mat in tomato grown in rockwool slabs – observation study, 2008

Treatment	Mean root-mat severity (0-5)				Number of dead plants
	21 May	21 June	28 July	2 Sep	10 Nov
1. Untreated	0.35	0.49	0.49	0.62	10
2. Gliomix	0.26	0.43	0.44	0.52	7
3. Biomex	0.21	0.34	0.35	0.47	4
4. Trianum P	0.10	0.12	0.10	0.12	0
5. Untreated	0.12	0.14	0.14	0.15	0
Significance (40 df)	-	0.012	0.012	0.009	-
LSD	-	0.25	0.28	0.33	-

Replicated experiment

Disease incidence increased between April and May 2008 and then remained around this level for the rest of the season. Mean disease severity increased between July and November, largely due to an increase in plants from category 2 (definite root-mat) to category 4 (collapse of root-mat roots). None of the plants died. Neither Ecoguard nor Rhizopro significantly affected the incidence or severity of root-mat at any of the assessments (Tables 3-5).

An assessment of roots within the slab at the end of cropping found no significant differences between treatments in the incidence or severity of root-mat (Table 3 & 4).

Table 3: Effect of two *Bacillus* preparations on occurrence of root-mat in tomato grown in rockwool slabs – replicated experiment, 2008

Treatment	Mean % cubes affected (index 2 or greater)					
	24 Apr	21 May	21 June	28 July	2 Sep	3 Nov
1. Untreated	6.9 (2.3)	14.7 (5.1)	11.8 (4.2)	12.9 (4.3)	12.9 (4.3)	13.4 (4.7)
2. Ecoguard	1.6 (1.3)	6.3 (3.9)	2.6 (2.4)	4.2 (2.9)	4.2 (2.9)	4.7 (3.3)
3. Rhizopro	2.6 (1.7)	5.7 (3.8)	5.2 (3.3)	5.2 (3.2)	5.2 (3.3)	5.1 (3.4)
Significance	NS	NS	NS	NS	NS	NS

( ) – standard error; NS – no significant differences

Table 4: Effect of two *Bacillus* preparations on root-mat severity in tomato grown in rockwool slabs – replicated experiment, 2008

Treatment	Mean disease severity (0-5)					
	24 Apr	21 May	21 June	28 July	2 Sep	3 Nov
1. Untreated	0.25	0.49	0.34	0.38	0.58	0.67
2. Ecoguard	0.09	0.26	0.08	0.18	0.19	0.20
3. Rhizopro	0.11	0.20	0.13	0.19	0.24	0.24
Significance (12 df)	NS	NS	NS	NS	NS	NS

NS – no significant differences

Table 5: Effect of two *Bacillus* preparations on root-mat on slabs at the end of cropping – November 2008

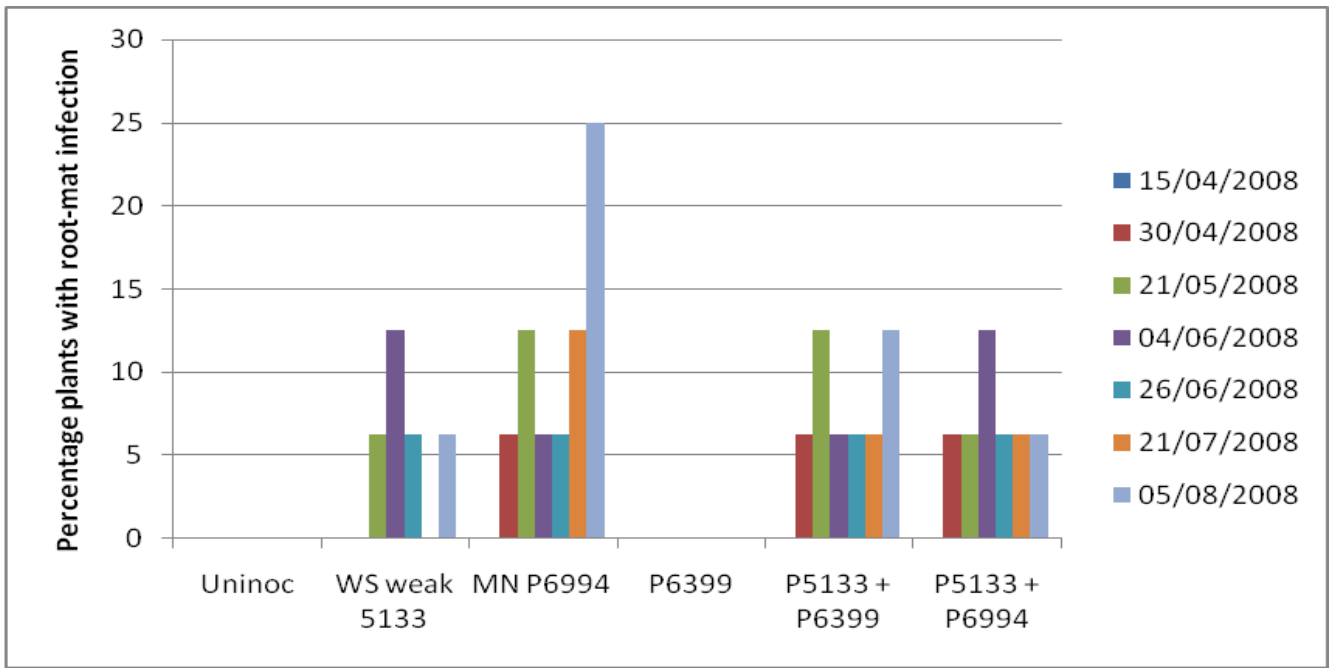
Treatment	Mean % plants affected	Mean severity (0-3 index)
1. Untreated	14.7 (6.1)	0.46
2. Ecoguard	11.2 (6.0)	0.34
3. Rhizopro	9.0 (5.5)	0.23
Significance (12 df)	NS	NS

( ) – standard error; NS – no significant differences

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

The small-scale glasshouse house trial carried out in 2008 at STC unfortunately suffered from a lack of good symptom development as has been seen in other trials. Although some slight symptoms were observed, the severity did not increase and only a few suspect, upright roots were seen and no block swelling developed. Figure 2 shows the percentage of plants in each treatment (out of a total of 16) which showed slight root symptoms at each assessment date.

Figure 2. The incidence of root-mat affected plants in the cross-protection experiment (shown as percent infected)



No root-mat was observed in the uninoculated plants, nor in those which had been inoculated with isolate P6399 – the supposedly virulent form of the Ri-plasmid isolated back in 2006. In all the remaining treatments only 1 or 2 plants showed symptoms (4 in the case of P6994), though this is insufficient to draw firm conclusions regarding possible cross-protection effects.

Results of additional studies carried out in 2008

<u>Objective e:</u> Effect of inoculation timing on symptom development

In the study designed and conducted between December and August 2008 root-mat symptoms first appeared, albeit at low levels, on a few plants following inoculation with isolates P6994 (MN) and P6399 (WS) 4 weeks post-sowing (15th April – some 8 weeks after inoculation) see Figures 3 & 4.

Figure 3. Mean incidence of root-mat symptoms in inoculation timing study in 2008

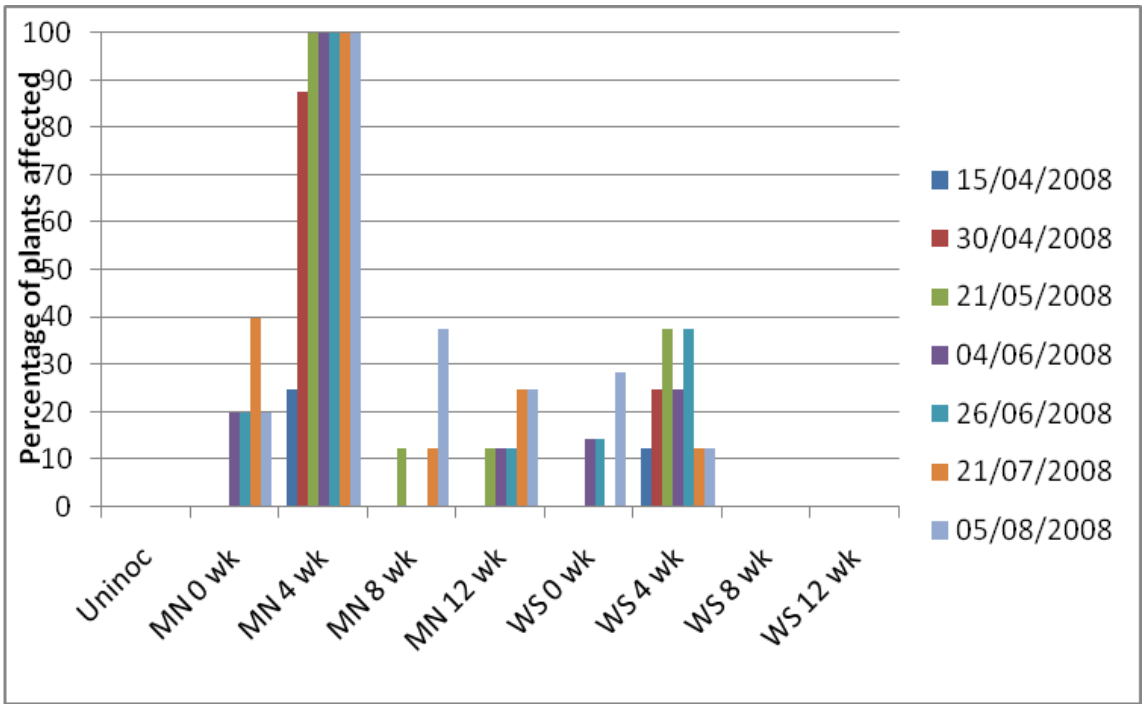
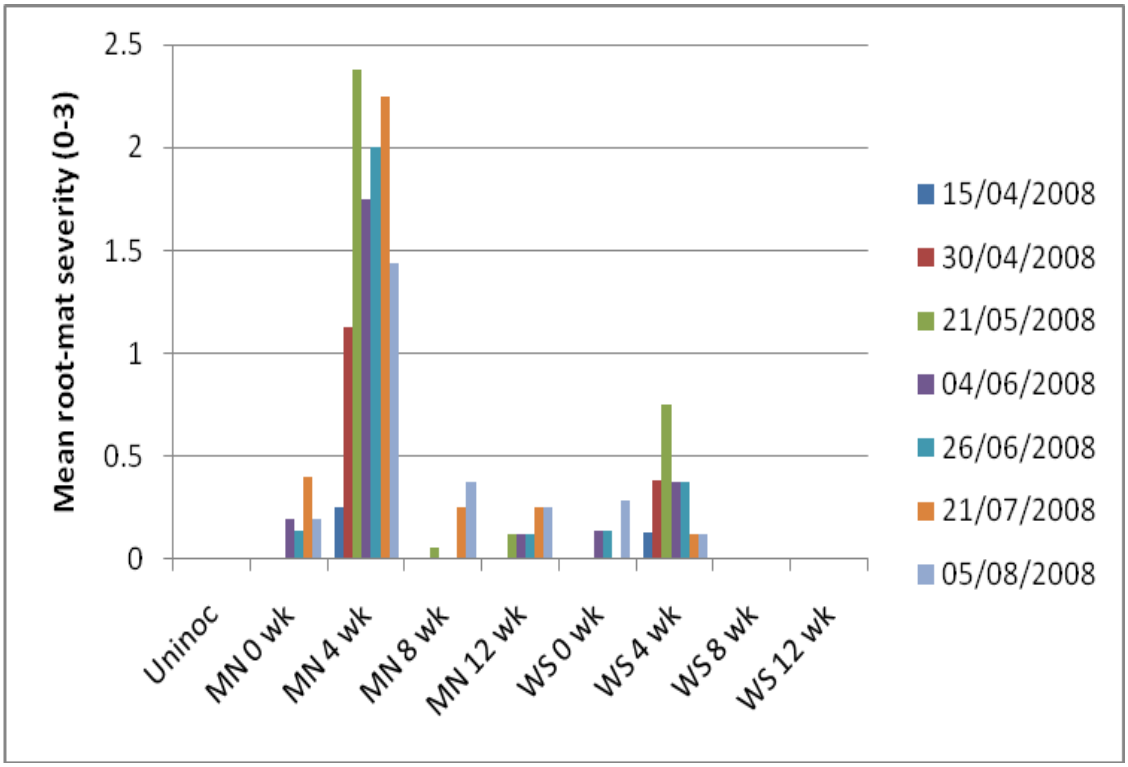


Figure 4. Mean severity of root-mat symptoms in inoculation timing study in 2008



In later assessments, both the incidence and severity of the disease increased by largely in the plants inoculated 4 weeks post-sowing. A relatively low level (severity index 1) of symptoms were seen in a number of the other inoculation timing treatments later in the study. The peak of symptom expression was seen around the end of May, after this time, those plants with the greatest root proliferation

succumbed to a root infection caused by a *Fusarium* sp. and the roots started to wither eventually resulting in death of the more severely affected plants (Plate 5).

Plate 5. Plant with root-mat symptoms (severity score 3) in the inoculation timing experiment 2008

The results from this replicated study certainly indicate that inoculation timing is extremely important for symptom development to occur. It suggests that initial infection with root-mat may well be occurring at an early stage e.g. during propagation, and therefore any potential treatment options e.g. biological products should be being applied during the critical period from sowing to planting, and perhaps beyond, to maintain protection. This data potentially explains the relatively poor symptom development in earlier trials as they tended to be inoculated post-planting when the plants appear to be less susceptible. We are now also sure that plants carrying the plasmid do not automatically develop root-mat symptoms and it seems that the plasmid needs certain 'triggers' to switch-on the root proliferation genes. This makes it difficult to complete robust trial consistently.

Objective f: Monitoring development of root mat in space and time

Root-mat symptoms were first observed in the allocated crop area in April. The proportion of affected slabs increased from 2.5% on 15 April to 11.5% on 21 June and decreased slightly thereafter (Figure 5).

There was no evidence of a greater occurrence of root-mat affected plants on slabs adjacent to the central concrete pathway than in the centre of pathways or at the glass wall end of rows (Table 9).

There was a trend of increasing occurrence of the disease in moving from the central pathway to the side wall (perhaps greater occurrence where more light was available).

The occurrence of groups of slabs affected by root-mat is shown in Table 10. Groups of two or more affected slabs along a row were less common than single affected slabs in assessments conducted in May (10 vs 29) and November (16 vs 44). Groups of two or more affected slabs across rows were considerably less common than single affected plants in both May (2 vs 53) and November (6 vs 89).

Figure 5: Development of root-mat over time in tomato, cv. Jack Hawkins, on rockwool slabs – 2008

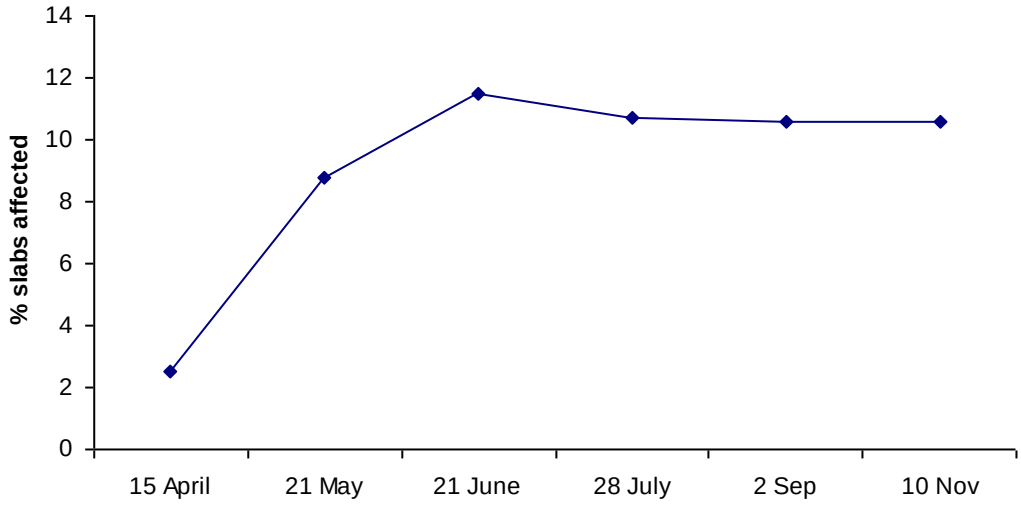


Table 9: Effect of slab position in a row on occurrence of root-mat in tomato on rockwool slabs in May and November 2008

Month assessed	Total number of slabs affected (of 220)		
	Adjacent to central pathway (slabs 1-10)	Middle of row (slabs 15-24)	Adjacent to site wall (slabs 32-41)
May	13	16	23
November	16	20	35

Table 10: The incidence of root-mat symptoms in different groupings of rockwool slabs - 2008

Number of affected slabs in the group	Number of slabs in each category			
	May		November	
	Along rows	Across rows	Along rows	Across rows
One	29	53	44	89
Two	5	2	9	6
Three	3	0	2	0
Four	1	0	3	0
Five	1	0	1	0
Six	0	0	1	0
Total	39	55	60	95

The monitored area consisted of 902 slabs (22 rows of 41).

The results observed here are consistent with previous observations (O'Neill, unpublished) that symptoms of tomato root-mat usually first occur in April, around 3-4 months after planting; also, that the incidence of the disease increases gradually for 2-3 months and little thereafter. It is unclear whether the increase in incidence of symptoms is due to spread between slabs, (i.e. a polycyclic disease with microbial movement between slabs), or to a difference in the latent period between infection and symptom development (i.e. a monocyclic disease).

The occurrence of runs of up to six adjacent slabs along a row with one or more plant affected by root-mat gives an impression of spread along a row. There is potential for movement of microorganisms between adjacent slabs in water films at the base of slabs and on roots that grow out of drainage slits in slabs and extend to the next downstream slab (M McPherson, unpublished) e.g. *Pythium* spp.. The

occurrence of runs of two or more slabs with symptoms of root-mat was much less common (by a factor of 3) across rows (i.e. when examining slabs in the same relative position in adjacent rows). There is less potential for movement of microorganisms in water or on roots across a row than along a row. These observations may indicate there is spread of the disease between adjacent slabs in a row early in the season. However, assuming all the plants in one propagation tray are planted on adjacent slabs along the same row, the occurrence of clusters of affected plants may alternatively reflect latent infection in a particular tray of plants at planting.

Objective g: Isolation of phage pathogenic to *Agrobacterium*

No phage pathogenic to *Agrobacterium* was detected during this study. Scientists at the phage institute in Tbilisi report that they found *Agrobacterium* phage to be very rare, and they had only had success with *A.vitis (pers. comm. R. Thwaites)*. However, this may be an area which warrants further work in the future.

Discussion

A frustrating lack of symptom expression during this three year study has impacted greatly on the results and conclusions that could be drawn. In years 1 and 2 of this study we only saw very weak symptom expression in the slow sand filter (SSF) trials at STC. During year 3 the filters were installed at 2 commercial nurseries in glasshouses which had a history of root-mat in previous seasons. However, at both nurseries root-mat symptom expression was very low in 2008 although tests on plants at Nursery B proved that the plasmid was present in the plants. The lack of symptom development in the SFF carried out in 2008 has made it impossible to draw any firm conclusions regarding the efficacy of any of the potential control treatments under investigation.

The biological product trial carried out at Nursery A did see some symptom development in plants during the 2008 season. Despite encouraging results from the small scale studies carried out at CSL in the previous year Gliomix (*Gliocladium catenulatum*) and Biomex SA (*Trichoderma* spp.) had little or no impact on the incidence and severity of infection compared to untreated plants, whilst Trianum P *(Trichoderma harzianum* strain T-22) potentially reduced infection to some degree. The two *Bacillus* products (Ecoguard and Rhizopro) did result in a slight reduction in symptom expression, however due to high levels of variability between the control plots and between replicates in all the experiments no significant differences were seen.

Small-scale experiments into the effect of microbial amendments on populations of rhizogenic *Agrobacterium* on both cucumber and tomato plants demonstrated that the Ri-plasmid could be detected on roots regardless of whether they had been treated with microbial products. While some reduction in symptom development was observed in cucumber, this was less evident in tomato. The apparent survival of the root mat pathogen in the rhizosphere of asymptomatic plants is intriguing, and may in part explain why eradication of the pathogen is so difficult. It seems likely that *Agrobacterium* can survive at low 'subclinical' levels and that, in the absence of competition from other microbes, persists to a sufficient degree to initiate infection under appropriate conditions. Results of the experiments on microbial amendments suggest that competition from biological control agents, notably *Trichoderma* and *Gliocladium*, may inhibit the onset of disease without necessarily completely excluding the pathogen from the rhizosphere.

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 particular root-stock choice non-viable economically. Until a more suitable and vigorous rootstock can be found it is not proposed to undertake further work on grafting.

The reported and observed natural decline of symptoms not only at the two commercial tomato nurseries involved in this study but also following a visit to a commercial cucumber nursery in 2008 is also of considerable interest and may have potentially commercial significance. Once again it is unclear what factors may have led to this situation. It is possible to speculate that this may have been due to a natural loss of pathogenicity/virulence of the plasmid, or an increase in suppressive or antagonistic micro-flora. Alternatively, environmental factors such as light intensity (reportedly lower in 2007 and 2008 than in previous years) may have had an effect on symptom development

Finally, the inoculation timing trial conducted in 2008 has provided important insight into the epidemiology of the disease and perhaps helps explain why many of the earlier trials failed to secure a robust and severe infection pressure. For future trials it appears to be very important to introduce the pathogen early (within 4-6 weeks of sowing) as the data suggests later inoculations are less successful.

Clearly we do not as yet have an answer to the problem of root-mat and its control though some progress has been made. Whilst the current study has not been able to demonstrate that a microbial diverse community helps prevent infection, neither has it refuted it and further studies are required, in situations of moderate-high disease pressure, to validate the hypothesis.

In the light of the inoculation timing experiment results it is recommended that the work is continued for a further year to investigate the use of microbial amendments during tomato and cucumber propagation (with early inoculation timings) to see if the disease can be alleviated in this way. It may also be appropriate to include the production of a fact sheet within an additional year's funding. The fact sheet would cover the findings to date and any relevant recommendations to growers as well as the findings of any additional studies.

Conclusions

- Large scale trials utilising slow-sand filters were conducted at STC and at 2 commercial nurseries. An absence of good or consistent root-mat symptoms resulted in a lack of data upon which to base firm conclusions as to the proposed efficacy of these systems to control root-mat.

- Molecular tests on non-symptomatic material collected from tomato have shown the presence of the Ri-plasmid in root material. The reasons for non-expression of symptoms are still not clear. However, it is now apparent that tomato root tissues may carry the plasmid and yet not be transformed, to proliferate roots.

- An inoculation timing experiment carried out at STC in 2008 has clearly demonstrated that plants are most at risk from infection at around 4 weeks post-sowing. This now demonstrates that any control mechanisms in tomato must be initiated during the propagation period to help maintain symptom-free plants.

- Initial laboratory-based experiments carried out on young tomato and cucumber plants suggested that Gliomix and Biomex SA had a beneficial effect when applied during and after propagation. However, later experiments on a commercial nursery carried out in the final year of the project did not support this finding. Instead the additional biological control product – Trianum P (*T. harzianum* strain T-22) did appear to reduce root-mat symptoms though due to variability in the control plots the result was not statistically significant.

- Where the disease was closely monitored in a commercial trial in 2008 root-mat symptoms first appeared in April 2008, 4 months after planting. The proportion of affected plants increased from April to June but not thereafter. The reason for symptom development being limited to this period of cropping is unknown. Possible explanations maybe the increased impact of countering factors such as lower light levels or the increase in natural microbial competition within the root-zone. There was a greater occurrence of root-mat symptoms in adjacent slabs along a row than across rows. This may be linked to the ability of the organism to spread along rows e.g. via irrigation run-off, or to positional factors.

Technology Transfer

- Root-mat update: Seminar to Wight Salads managers, Arreton Valley, Isle of Wight, 19th April 2007and 2008 (Tim O'Neill).
- A number of interested grower groups which visited STC during 2007 and 2008 had an opportunity to see the work and glasshouse trials in progress.

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; to Enza for providing the aubergine seed, and to Max Burton and Rob Waterhouse of Wight Salads Group for help with this project. The project team also extend the grateful thanks to the management and staff at both of the commercial nurseries who provided their crops, time and interest to help facilitate this trial.

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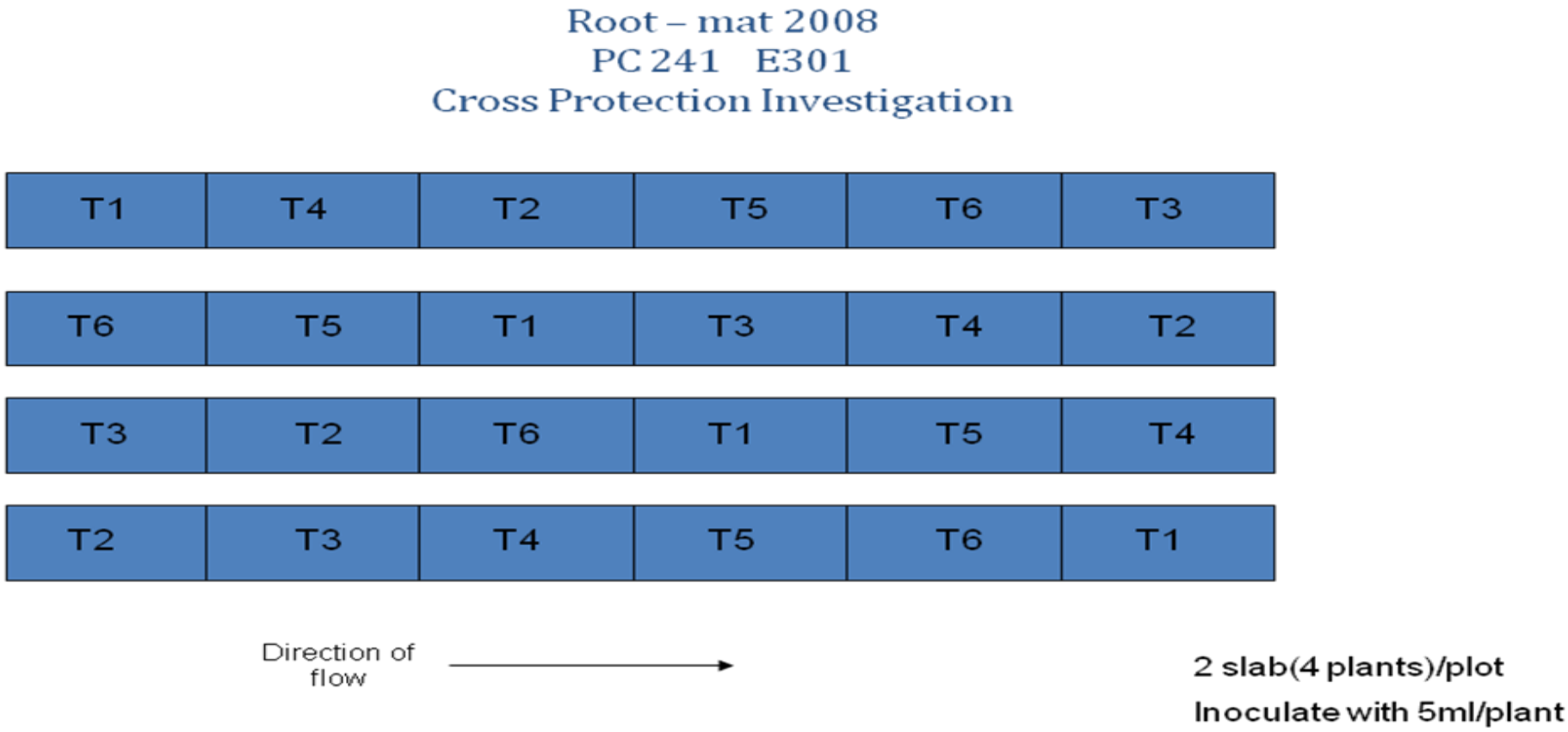
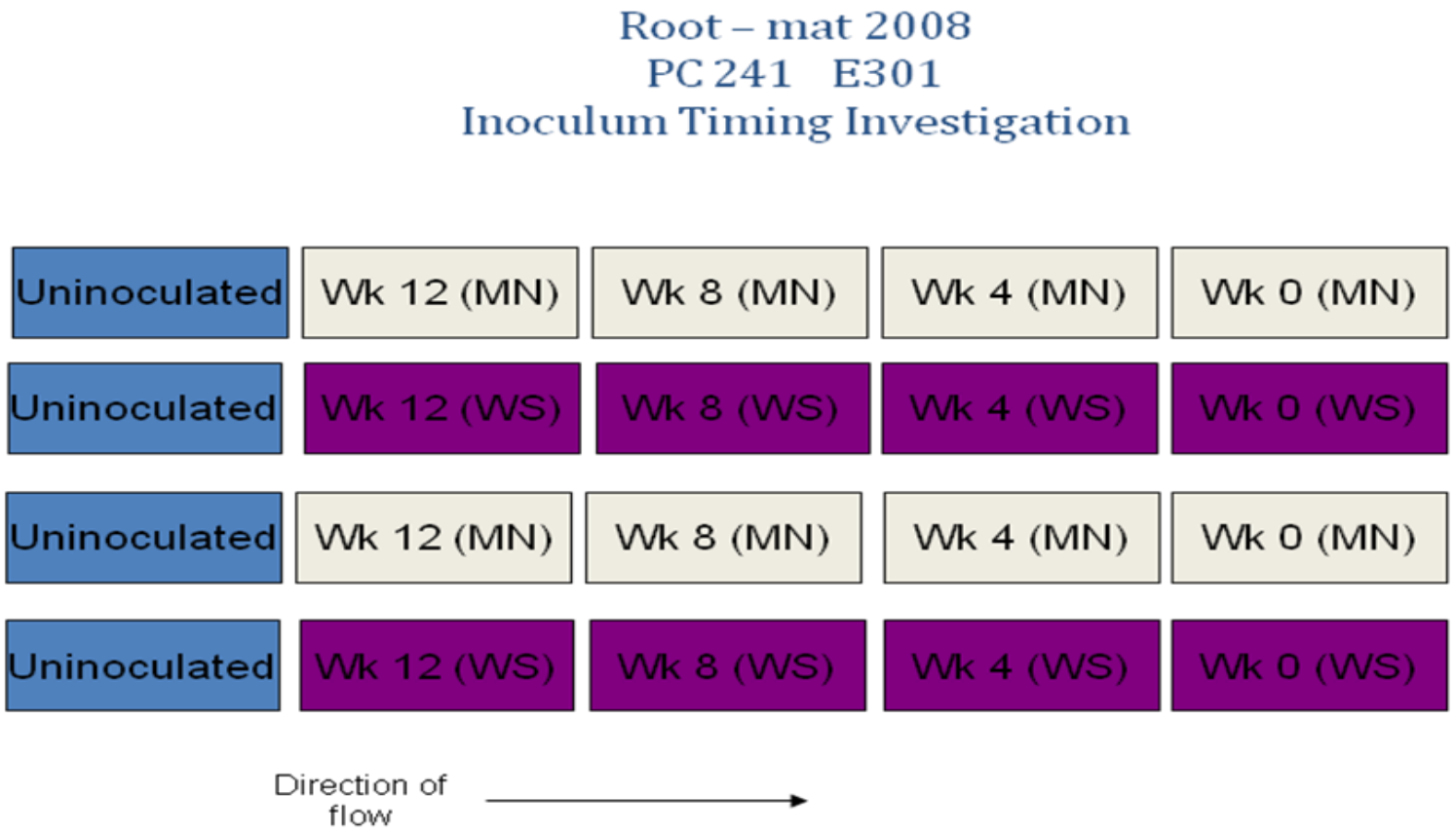
Appendix 1. Trial Plan for Cross protection experiment at STC in 2008



T1 Uninoculated control
T2 WS weak strain only
T3 MN P6994 only
T4 – WS virulent strain only P6399
T5 – WS weak + WS virulent
T6 – WS weak + MN P6994

Appendix 2. Inoculum Timing experiment trial plan – STC 2008



WS – Inoculum from WS Isolate P6399 (Claree 2006)

MN – Inoculum from MN P6994 (Elegance 2007)

2 slabs(4 plants)/plot