Project Title:	The development of a sustainable system for the prevention of root disease in recirculating hydroponic crops
HDC Project Number:	PC 60a
LINK Project Number:	P120
Report:	Final report, December 2000
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Location:	HRI - Stockbridge House & University of Hull
Date Project Commenced:	April 1994
Date Completion Due:	March 1999
Keywords:	Tomatoes, cucumbers, hydroponics, recirculation, disease, *Phytophthora, Pythium, Colletotrichum, Thielaviopsis, Phomopsis, Verticillium, Fusarium,* disease suppression, disinfection, pasteurisation, ozonisation, UV, micro-filtration, slow sand filtration, per-acetic acid.

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The results and conclusions in this report are based on a series of experiments over 5 years. The conditions under which the experiments were carried out and the results 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 product recommendations.

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BACKGROUND AND OBJECTIVES

Previous MAFF funded studies have demonstrated that several important root pathogens are readily disseminated in nutrient solution that has been collected and re-used, for salad crop production in the UK. More recently, HDC funded experiments have indicated that disinfection treatment of the nutrient solution can alleviate, and in some cases, prevent spore dispersal (see HDC project PC 60). At the same time, it has occasionally been observed that, in the absence of disinfection, root pathogens appear to be suppressed biologically to a greater or lesser degree. However, the precise mechanisms for the suppression are not understood.

Further work was commissioned and the broad objectives of the LINK project were:

a) *To further validate currently available water disinfection treatments differentiating between those which provide a complete (or near) kill of all microorganisms (ie active disinfection) and those which selectively eliminate pathogen spores whilst retaining biological activity in the root environment (passive disinfection).*

b) *To undertake a quantitative evaluation of the microbial components of hydroponic tomato and cucumber crops in a disease-free environment and to determine whether species diversity is consistent between crops and if any shift in microbial population occurs following artificial inoculation with important root pathogens (links with PhD studentship).*

c) *To investigate what effect disinfection of the hydroponic solution has on the microbial components of the root environment.*

d) *To investigate the potential of any species recovered for their potential as antagonists against the primary root pathogens of tomato and cucumber.*

e) *To investigate the potential for monitoring population shifts of key indicator species in the root environment microflora as a technique for the early detection of root infection and ultimately as an indicator of whole crop health.*

A key aspect of this project was the appointment of a PhD studentship linked to the University of Hull to undertake the fundamental studies in the project, utilising where possible, the trial facilities at HRI Stockbridge House on a seasonal basis.

Specific objectives of the PhD studentship were:

i) *To screen a range of bacterial isolates recovered from hydroponic solution from tomato and cucumber for anti-fungal activity in-vitro.*

ii) *To characterise, as far as possible, the predominant bacterial isolates exhibiting anti-fungal activity from the recirculating hydroponic solution of both tomato and cucumber crops.*

iii) *Determination of the occurrence of secondary metabolites from selected bacterial isolates and characterisation of the mechanism(s) of anti-fungal activity.*

iv) *To evaluate the most promising isolates for anti-fungal activity in small-scale experiments in-vivo.*

v) *To devise a simple bioassay procedure for quantifying the suppressiveness of hydroponic solution from tomato crops.*

vi) *To evaluate hydroponic solution from commercial tomato nurseries for the presence of suppressive activity.*

vii) *To conduct preliminary tests for direct host effects including phytoalexin production, other anti-microbial compounds and development of systemic acquired resistance (SAR) in host plants.*

SUMMARY OF RESULTS

Disinfection of nutrient solutions in recirculating hydroponic systems

Numerous commercial disinfection systems were installed at HRI Stockbridge House, including a Priva UV 'Vialux' unit, a Brinkman 'Drainheater' pasteuriser, an Ozotech ozonisation unit, a Memcor micro-filtration unit, a Solvay-Interox Proxitane dosing unit and latterly an 'in-house' designed slow sand filtration unit. In initial evaluations to compare their performance against the primary fungal pathogens of tomato and cucumber they all worked well in that crop losses due to severe disease in closed systems was minimal. Specific details on the efficacy of each treatment is provided below.

<u>Proxitane 1210 (per acetic acid/hydrogen peroxide)</u>
Treatment with Proxitane 1210 (per-acetic acid/hydrogen peroxide), whilst effective in preventing pathogen spore dispersal, was problematic due to (i) the development of *Trichoderma* spp. in solution which caused blockage at the drip lines and (ii) phytotoxicity symptoms, particularly on cucumbers. It is possible that an engineering solution could be found to resolve these problems, though this was not achieved in the time course of this study. Therefore, per-acetic acid based products cannot be recommended for use in hydroponic systems at the present time.

<u>UV and Ozone</u>
Treatment with UV and Ozone were both effective in preventing economic crop loss in the experiments reported though evidence was gained to suggest that both *Pythium* and *Colletotrichum* continued to be disseminated after disinfection. The lack of any appreciable adverse effect on the trial crops suggests either that the dissemination occurred late in the cropping cycle or that the inoculum levels being disseminated were very low and the disease progression therefore delayed. This problem could potentially be rectified by increasing the applied dose of either UV or Ozone, though clearly this would influence the economics of the treatment relative to other systems. It is also of some concern that, particularly with UV, there are numerous systems on the market and these are very different from each other; some more sophisticated than others. The Priva 'Vialux' system evaluated here is highly sophisticated with all necessary safety devices to ensure an appropriate dose is consistently applied. The

efficiency of the UV lamps deteriorates over time due to a variety of factors but particularly deposition eg iron chelate on the quartz tube. Unless there is an automatic wipe facility on the unit (or access to do this manually) performance of the UV equipment will deteriorate over time.

In the case of Ozone disinfection, we noted that the ozone demand for cucumbers was significantly greater to that for tomatoes. It was assumed that the increased root development and activity of cucumbers liberated greater quantities of organic root exudates, thereby increasing the ozone demand. Providing an ozone residual is determined for the specific crop this does not pose a particular problem (apart from increased operational costs). However, it should also be considered that the ozone demand could fluctuate and potentially increase considerably over the time course of the season due to a variety of factors, not least the root volume, the rate of decay of root tissues, the degree of organic matter contamination (open vs. closed channels for collection) and the rate of build-up of algae and other organic constituents of the hydroponic environment. Regular checks for an ozone residual should be made to maintain the efficiency of disinfection. It should also be noted here that ozone is a particularly toxic gas and there are considerable health and safety implications for its use commercially.

Pasteurisation

The Brinkman pasteuriser proved to be one of the most reliable disinfection techniques evaluated. It halted the spread of the introduced pathogens very effectively and was extremely reliable from a maintenance standpoint. Also, the technology is easy to understand and in theory provides flexibility to adjust the operating temperature depending on the specific pathogen(s) being targeted. For example, zoospores of *Pythium* are likely to be disrupted at temperatures well below 95°C and if this is the only problem on the nursery considerable savings could be made. In contrast, if chlamydospores of a pathogenic *Fusarium* sp. are prevalent causing disease then a higher temperature is needed.

This fundamentally is one of the greatest difficulties associated with such 'active' disinfection technology because commercially it is difficult to predict precisely what pathogens will be prevalent in a particular year. By the time symptoms become evident in the aerial canopy dissemination is likely to be widespread and the damage has already occurred. It is advisable therefore to maintain a fairly high dosing level (heat, UV, Ozone) as an 'insurance' against the unexpected.

It should also be pointed out that little work has been conducted under commercial conditions to evaluate disinfection systems against bacterial (eg *Rhodococcus (Corynebacterium micheganense)* or virus pathogens (eg Pepino Mosaic Virus, Cucumber Green Mottle Mosaic Virus). However, whilst these pathogens may be disseminated via the recycled irrigation water they will also be readily transmitted mechanically in the aerial canopy and therefore the value of solution disinfection is questionable.

Micro-filtration

Micro-filtration proved to be very effective in the experiments. However this system is relatively expensive in terms of capital and running costs; particularly as there is no assurance of increased yields, improved quality etc. All these disinfection systems can only be regarded as an insurance treatment against crop loss. Filtration techniques have previously been regarded as impractical due to problems with pore clogging following long- term use. This aspect of the technique could not be fully evaluated here, as the throughput of hydroponic solution was relatively low compared to anticipated use in a commercial environment. Therefore any grower contemplating 'closed' system technology utilising this type of disinfection should investigate this aspect carefully with the manufacturer before investing heavily. Growers would also need to look carefully at all the costs associated with installing, running and maintaining the various types of disinfection equipment in the long-term.

Studies of the biology of nutrient solutions in closed systems

Over 3 seasons from 1996 to 1998 a series of experiments were undertaken in an attempt to study the phenomenon of naturally occurring disease suppression in closed hydroponic systems. Experiments to monitor 'open' and 'closed' systems and 'active' and 'passive' disinfection treatments were undertaken in large scale replicated studies. The introduced pathogens of *Pythium* spp and *Phytophthora* spp. were allowed to disseminate freely in advance of any imposed treatments so that comparisons of suppression development could be made between 'open' and 'closed' regimes and between 'active' and 'passive' disinfection techniques. At this juncture, Slow Sand Filtration was used for the first time in the project.

The trials provided strong evidence to demonstrate the occurrence of microbial suppression, particularly following passage through a slow sand filtration unit. Specific microbes sourced from suppressive hydroponic solution were shown to be antagonistic to the primary root pathogens such as *Pythium* and *Phytophthora* spp. and various metabolites liberated into solution help to account for the observed antagonism. The antibiotic 2.4-diacetyl-phloroglucinol was identified in suppressive solution liberated by specific bacteria. This undoubtedly is one of the underlying mechanisms to account for the phenomenon.

The siderophores, Pyoverdine, Pseudobactin and Pseudobactin A were also identified in hydroponic solution liberated by various bacteria and this provides a second mechanism by which root pathogens may be suppressed in hydroponic solution. Siderophores are known to inhibit fungal mycelial growth and germ tube elongation (rather than inhibiting zoospore germination). Separately, cultures of specific microbial components were sent to another PhD student at the University of Gent, Belgium to determine whether any produced salicylic acid. This compound is known to act as a trigger for systemic acquired resistance, a host resistance mechanism to combat various plant diseases. At least one of the isolates recovered from suppressive solution was subsequently reported to produce salicylic acid. This provides a third mechanism to account for the observed phenomenon.

The results suggested that a suppressive potential may develop in 'closed' systems irrespective of any introduced pathogens and that the development of suppression was progressive over the season. Strong evidence was gained to support the hypothesis that passive disinfection technology ie slow sand filtration, was beneficial in generating a strong microbial suppression. Conversely, some limited evidence was gained to suggest that the enhanced effect was lost when 'active' disinfection systems such as UV, ozone or heat, were used. Certainly 'active' disinfection disrupted microbial populations and tended to lead to single bacterial populations in the treated water. It is therefore possible that where 'active' disinfection systems are deployed much of the antagonism from the diverse microbial population would be lost.

Finally, and concurrently with this work, Stanghellini *et al* (1997) were able to show that microbially liberated biosurfactants could lyse zoospores of oomycete fungi in hydroponic systems. Whilst this was not fully demonstrated to occur in the experiments reported here, it does potentially provide yet another mechanism to account for the suppressive effects.

If the various mechanisms associated with microbial suppression can be effectively harnessed then clearly there is an excellent opportunity for the development of a sustainable system for control of root disease in recirculating hydroponic systems. The variability observed in this work is consistent with that of most biocontrol work and is due largely to our poor understanding of the specific identity, requirements and interactions of the various antagonists. Further R&D is needed at a strategic level in order to truly exploit the microbial antagonism that exists within recirculating hydroponic systems.

ACTION POINTS FOR GROWERS

- Growers considering 'closed' production systems for glasshouse cropping should install an 'insurance' disinfection treatment to minimise the risk of crop loss due to root diseases.

- Where 'closed' systems are deployed every effort should be made to minimise rooting out into the solution collection channel as pathogens will be disseminated irrespective of any applied disinfection treatment.

- Active disinfection treatments including UV, ozone or heat (pasteurisation) are effective in providing disease control in closed production systems.

- When selecting a UV disinfection system, ensure that the system has all of the necessary monitoring and safety devices to deliver an accurate and appropriate dose. Maintenance of the UV lamp is important; an automatic wipe clean facility or routine manual cleaning is required.

- If using ozone for disinfection, regular checks are needed of the ozone residual in the system to ensure maximum efficacy.

- While micro-filtration and pasteurisation performed well in these trials, growers should seek evidence of performance on a commercial scale prior to investment.

- Passive disinfection technology using slow sand filtration offers a low cost option for disinfection but throughput is an issue and specific management expertise is needed (see HDC project reports for HNS 88, HNS 88a and HNS 88b).

- Growers should <u>avoid</u> the use of per-acetic acid, and combinations thereof, as a technique for water disinfection where drip irrigation systems are deployed. The development of *Trichoderma* spp. in the lines could be very costly.

- There are numerous microbial inoculants currently on the market all with various claims for potential benefits for hydroponic crops, though few claim disease control. If your supplier is unable to provide specific details in this regard it would be advisable to avoid use.

- From this work, and that elsewhere in MAFF project HH1724SPC, there are indications that a rapid replenishment with 'fresh' nutrient solution could trigger mass zoospore germination of *Pythium* and *Phytophthora* species with extensive root infection. Growers of NFT crops should therefore consider the merit of a gradual replenishment to minimise this risk.

ANTICIPATED BENEFITS FROM THE STUDY

This work provides growers with much more confidence in the effectiveness of disinfection technology should they wish to adopt 'closed' system technology in glasshouse crops production. Most importantly, the project has had a major impact in increasing the industry's awareness of the complex microbial interactions taking place in these hydroponic crops.

The work has identified that the root environment in hydroponic crops, however inert, would appear to be extremely complex and in this respect the project has perhaps raised more questions than provided answers. Further research will undoubtedly be needed to attain the goal of fully closed systems for hydroponic crop production.