FINAL REPORT

Project Number:	PC60
Project Title:	Evaluation of disinfection systems for the control of root pathogens in hydroponic crops of tomato and cucumber grown using recirculation technology
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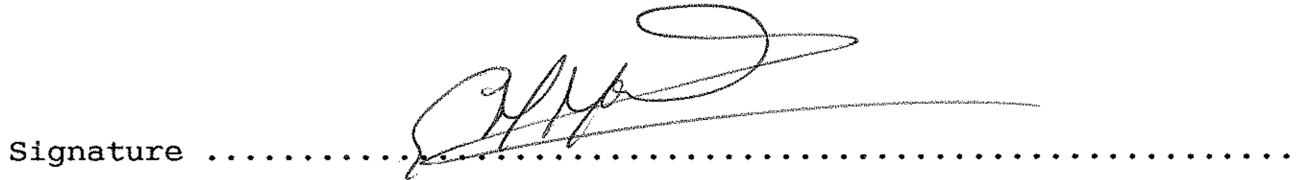
Date Project Commenced:	1 April 1992
Report Date:	31 March 1996
Key Words:	Hydroponics, tomato, cucumber, recirculation, disinfection, root disease, *Pythium*, *Phytophthora*, *Fusarium*, ELISA, bioassay
Distribution:	HDC (2) Dr N Dungey (1) Dr G M McPherson (1) HRI Archive (1)

The results and conclusions in this report are based on a series of experiments. 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.

Authentication

I declare that this 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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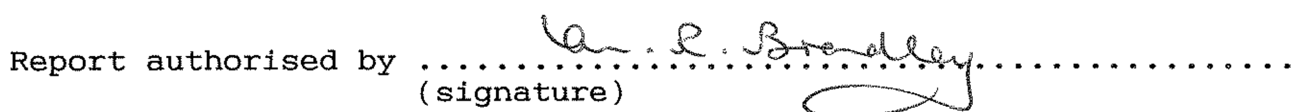

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RELEVANCE TO GROWERS AND PRACTICAL APPLICATION

It is anticipated that the protected salads industry will be under increasing pressure to conserve water and to avoid pollution of ground-water and the wider environment. Collection and re-use of the excess irrigation applied to hydroponic crops is desirable but the threat of root disease being spread in the recirculated water is a major barrier to its adoption.

Previous MAFF funded studies in HRI have demonstrated that many fungal root pathogens are disseminated in the irrigation water and pose an increased threat to the crop.

This project was designed to determine whether it would be feasible to disinfect the collected hydroponic solution prior to re-use and thereby prevent pathogen dissemination.

The main findings were:

- Root rot caused by *Phytophthora cryptogea* was effectively prevented from disseminating in tomatoes using a Priva UV 'Vialux' disinfection system and an Alfa Laval pasteurisation system.

- The design of the nutrient solution collection system is important to prevent rooting out of the slabs and therefore avoid disease spread down the row, prior to disinfection.

- Yield loss due to *P. cryptogea* occurred in this trials series but was significantly lower than anticipated, even though the pathogen was dispersed to greater than 90% of the crop in the inoculated control plots.

- Root rot caused by *Pythium aphanidermatum* was effectively prevented from being disseminated widely in cucumbers using a Priva UV 'Vialux' unit, a Brinkman 'Drainheater' pasteuriser, an Ozotech ozone generation unit and a Memcor micro-filtration unit.

- Localised pockets of infection by *P. aphanidermatum* did occur in the disinfected plots but it is considered that aerial spread by sciarid and/or shore flies may have been responsible for this.

- Whilst widespread infection by *P. aphanidermatum* occurred in the inoculated control plots in the cucumber trial, yield loss was again lower than anticipated and the crop appeared able to 'recover' from this aggressive root pathogen, as evidenced by the lack of characteristic symptoms in the aerial plant parts in the later stages of the trial.

- *F. oxysporum* f.sp. *lycopersici*, cause of vascular wilt of tomato was well controlled by the various disinfection equipment under test in the third experiment. Small foci of infected plants were found in treated plots, but due to the severe symptom expression on aerial parts of the plant ie sporulating lesions, it is regarded that these small foci may have been due to air-borne rather than water-borne transmission.

- The nutrient status of the hydroponic solution was altered following disinfection treatment. Fe in particular was oxidised following UV and ozone treatment. The nutrient loss following disinfection was not regarded to be critical as it only affects the 30-40% run-off (collection) solution.

- As a result of these trials growers of both tomato and cucumbers could confidently adopt recirculation technology and maintain freedom from the aforementioned pathogens, providing one of the disinfection systems was installed and functioning effectively.

- In cucumbers, further attention needs to be paid to the role of sciarid and/or shore flies in the spread of fungal pathogens in the cropping house. Measures for their effective control need to be identified.

- The observations in both tomato and cucumber crops grown using recirculation of delayed establishment or even 'recovery' from disease in the absence of disinfection had led to the concept that disease suppressive factors/agents are present within the hydroponic solution.

- If disease suppressive factors are present in solution then the choice of disinfection system may be more critical than previously supposed. 'Active' disinfection eg. heat, UV and ozone are likely to negate any suppressive activity in the solution. 'Passive' systems, eg. filtration, are likely however to retain these benefits.

- This project had identified an opportunity for the development of a sustainable system for the control of root pathogens in hydroponic crops and a LINK project has been secured to continue work in this area. It is recommended that growers should, where possible, delay installation of disinfection systems until further information is available.

INTRODUCTION

In the UK most hydroponic crops, grown under protection, currently utilise a run-to-waste system of culture. Crops are irrigated to excess (ca. 25-35%) to counter both the potential variation in the irrigation supply and individual plant's demands for water; the excess nutrient solution being discarded. Due to financial and environmental constraints the UK industry is eager to adapt growing systems to utilise recirculation technology to ensure that the nutrient solution is retained and re-used. However, the perceived threat from root pathogens, disseminated in the recirculating solution, and occasional widespread root disease where recirculation has been tried by commercial growers, currently prevents widespread adoption of the technology.

Several workers (Paludan, 1985; Thomlinson & Faithfull, 1979; Jenkins & Averre, 1983; Bates & Stanghellini, 1984; Daughtrey & Weller, 1992; Rattink, 1983 & 1991; Sanogo & Moorman, 1993; and Stanghellini & Rasmussen, 1994) have already demonstrated that certain root pathogens can be effectively disseminated in hydroponic solution; though other trials have provided conflicting evidence (Staunton & Cormican, 1978; George *et al*, 1989; and Rattink, 1990).

Replicated studies at HRI Stockbridge House and Efford in tomato, cucumber and ornamental crops grown using ebb-flood techniques have clearly demonstrated that root pathogens, eg. *Phytophthora cryptogea, Pythium aphanidermatum, Thielaviopsis basicola* and *Fusarium oxysporum*, are readily disseminated in recirculating hydroponic solution. Interestingly, whilst pathogen dissemination has occurred disease progress and subsequent yield loss has been lower than anticipated and it has been suggested that a 'natural' or 'biological' suppression occurs in the recirculating solution (McPherson *et al*, 1995).

A series of legislative measures, introduced in Holland in the form of the Multi Year Crop Protection Plan, set target dates for their adoption (Anon, 1990). By the year 2000 all glasshouse crops must be grown in closed cultivation systems to prevent surface and ground water contamination. It is anticipated that European legislation or marketing pressures from the Dutch industry may persuade growers elsewhere, including the UK, to develop closed systems. However, the continued availability of effective pesticides to counter the increased spread of root pathogens, is in some doubt and before adopting recirculation systems UK growers would need some assurance that alternative strategies for prevention of widespread pathogen dispersal and yield loss due to disease were available.

In the Netherlands and elsewhere, research has been conducted to evaluate the potential of various disinfection techniques including, heat, UV, ozone, ultra-filtration, micro-filtration, slow sand filtration, iodine and activated hydrogen peroxide (Stanghellini *et al*, 1984; Runia, 1988, 1994, 1994a, 1995; Runia *et al*, 1988; Goldberg *et al*, 1992; Wohanka, 1990; Wohanka, 1993; Vanachter *et al*, 1988) though few studies have been conducted under semi-commercial conditions. The results from studies up to 1992 were summarised by O'Neill and Berry in HDC report CP4.

The aim of the present study therefore was to conduct a series of trials under semi-commercial conditions to determine whether various disinfection techniques (heat, UV, ozone, filtration) could effectively prevent widespread dispersal of the primary root pathogens of tomato and cucumber crops following their artificial introduction.

MATERIALS AND METHODS

Location

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Plant Raising

All plants for the trials were raised from seed at HRI Stockbridge House. Seed was sown directly into propagation blocks in a quarantine area free from root pathogens. To ensure plants remained free from disease throughout the propagation phase, they were raised off the floor on 5 cm deep polystyrene panels overwrapped with new polythene. Plants were checked weekly for the presence of root discoloration and any suspect roots examined microscopically. Access to this site was restricted and foot dips deployed at the entrance to minimise pathogen/pest introduction. Plants were subsequently transferred to the growing house, taking care to ensure freedom from contamination by lining all trays used for transportation with new polythene.

Disinfection Installations

All the disinfection equipment was installed in conjunction with the manufacturers. All trials were designed such that cross-contamination of solution between treatments could not occur. Because of the relative low volume of run-off from plots in the individual treatments it was necessary to batch treat the solution. Solution collected from each plot was collected in a tank (collection tank) at the end of each row and returned to a larger 1000 litre holding tank (pre-treatment tank) when full. It is important to note that solution collected from each replicate plot in each treatment was pooled in this pre-treatment tank and therefore they do not represent true replicates. This fact should be considered at all stages when interpreting the results from the trials series. Once the pre-treatment tanks were full disinfection

was triggered on a batch basis, the solution passing to a further 1000 litre (post treatment) tank after disinfection. From here the solution was taken and mixed with fresh water and nutrients via a series of feed units prior to returning to the same area of crop in the trial. All the units were alarmed and HRI staff were on call 24 hours/day, seven days/week to respond to any breakdowns/ difficulties. This was necessary to ensure untreated solution did not bypass the disinfection process if the equipment failed to function. Full details of the various disinfection equipment is included in Appendix III. See also Figures 1-5 in Appendix I.

Nutrient Solution Monitoring

There are a few reports in the literature regarding potential detrimental effects of solution disinfection on nutrient status. For this reason random analyses were conducted during the trial period to determine their effect on various elements. Results of these analyses are presented for information.

Trial Details

Trial 1: January to November 1992

Crop: Tomato

Cultivar: Liberto

Site: House 5 (1000 m^2 venlo unit)

Design

Randomised block with four main treatments sub-divided into two channel (culture) systems. Four replicates for each of the inoculated treatments (including the disinfection treatments) and three each for the uninoculated controls. Each plot comprised of a double row with 60 plants per plot in total.

Treatments

1. Uninoculated control. Channel system 1. (3 replicates only).
2. Uninoculated control. Channel system 2. (3 replicates only).
3. Inoculated control. Channel system 1.
4. Inoculated control. Channel system 2.
5. Inoculated Channel system 1. Treatment of recirculation solution with a Priva UV "Vialux" unit at a dose of 100 mJ/cm^2.
6. Inoculated Channel system 2. Treatment of recirculation solution with a Priva UV "Vialux" unit at a dose of 100 mJ/cm^2.
7. Inoculated Channel system 1. Treatment of recirculation solution with an Alfa-Laval pasteuriser operating at a temperature of 95°C for 30 seconds.

8. Inoculated Channel system 2. Treatment of recirculation solution with an Alfa-Laval pasteuriser operating at a temperature of 95°C for 30 seconds.

Channel Systems (Trial 1 only)

To evaluate the potential spread of the introduced pathogen and the performance of the disinfection techniques it was imperative that a system of culture was identified which avoided rooting out from the growing medium into the run-off solution beneath. In this trial two systems were evaluated.

Channel System 1: comprised a semi-rigid through with bridges spanning it, onto which were placed the rockwool slabs. The bridges were positioned such that there were 3/slab (1 central and 1 at either end). The rockwool slabs were slit in the normal manner and the excess solution collected in the trough below and subsequently in a tank at the end of the row before recirculation.

Channel System 2: comprised a semi-rigid trough into which were placed two lengths of polystyrene (4 x 4 cm). The troughs were overlain with polythene to allow a small gutter in the centre of the row. The rockwool slabs were placed on the polythene on top of the polystyrene and slit in the normal manner. The polythene was also slit to ensure the solution ran through to the trough below and subsequently collected in a tank at the end of the row before recirculation.

Trial 2: January to July 1993

Crop: Cucumber

Cultivar: Corona

Site: House M20 (1000 m^2 modern glasshouse unit) and House 11 for disinfection installation only

Design

Randomised block with six treatments. Three replicates for each of the uninoculated and inoculated controls and two replicates for the remaining treatments. Each plot comprised of a double row each containing unwrapped rockwool slabs in 10 Brinkman aquatrays (3 plants/tray) with a total of 60 plants per plot.

Treatments

1. Uninoculated control. No treatment of the recirculating hydroponic solution.

2. Inoculated control. No treatment of the recirculating hydroponic solution.

3. Inoculated. Treatment of all the recirculating hydroponic solution with a Brinkman "Drainheater" pasteuriser operating at 95°C for a period of 30 seconds.

4. Inoculated. Treatment of all the recirculating hydroponic solution with a Priva UV "Vialux" unit providing a dose of 100 mJ/cm^2.

5. Inoculated. Treatment of all the recirculating hydroponic solution with an Ozotech ozoniser generating 4 mg ozone/litre water with a treatment time of 6 minutes to give a residual concentration of 0.5 mg ozone/litre water.

6. Inoculated. Treatment of all the recirculating hydroponic solution with a Memcor continuous flow micro-filtration unit providing a mean pore diameter of 0.2 microns.

Trial 3: August to November 1993

Crop: Tomato

Cultivar: Ailsa Craig

Site: House M20 (1000 m^2 modern glasshouse unit) and House 11 for disinfection equipment installation only.

Design

Randomised block with six treatments, replicated five times. Each plot comprised of a double row each containing unwrapped rockwool slabs in 10 Brinkman aquatrays (3 plants/tray) with a total of 60 plants per plot.

Treatments

1. Uninoculated control. No treatment of the recirculating hydroponic solution.

2. Inoculated control. No treatment of the recirculating hydroponic solution.

3. Inoculated. Treatment of all the recirculating hydroponic solution with a Brinkman "Drainheater" pasteuriser operating at 95°C for a period of 30 seconds.

4. Inoculated. Treatment of all the recirculating hydroponic solution with a Priva UV "Vialux" unit providing a dose of 100 mJ/cm^2.

5. Inoculated. Treatment of all the recirculating hydroponic solution with an Ozotech ozoniser generating 4 mg ozone/litre water with a treatment time of 6 minutes to give a residual concentration of 0.5 mg ozone/litre water.

6. Inoculated. Treatment of all the recirculating hydroponic solution with a Memcor continuous flow micro-filtration unit providing a mean pore diameter of 0.2 microns.

<u>Inspection Ports</u>

To provide static locations for monitoring pathogen dispersal inspection ports were prepared in advance in 10 evenly spaced slabs or troughs per plot and given a unique number. A 4 cm diameter core of rockwool was removed from the centre of each designated slab using a purpose-built tool. A 7.5 cm length of irrigation piping (4 cm diameter) was inserted into each port, the lower end cut obliquely to allow solution to flow freely into and out of the port. At regular intervals "bait" seedlings (tomato or cucumber) were placed in each port, left *in situ* for 48-72 hours, removed and placed into individual polythene bags for further testing (see Figure 6 in Appendix I).

<u>Pathogen Preparation and Inoculation</u>

Trial 1:

An isolate of *P. cryptogea* was recovered from infected tomato root tissue exhibiting characteristic symptoms and its identity confirmed in subsequent host tests. The pathogen was raised on potato dextrose agar (PDA) for 72 hours before being used as inoculum in this trial. All plots (excluding uninoculated treatments 1 and 2) were single point inoculated by placing 2 x 1 cm agar discs containing *P. cryptogea* into a previously designated inspection port, previously prepared in the centre of the slab, ensuring the discs were sited at the bottom of the port. Inoculation was carried out immediately prior to the first pick when fruit load was high. Pathogen establishment in the inoculated ports and potential dispersal to other ports in the plot were monitored using a tomato seedling bioassay and specific serological techniques (see below).

Trial 2:

An isolate of *P. aphanidermatum* was recovered from infected cucumber root tissue exhibiting characteristic symptoms and its identity confirmed in subsequent host tests. The pathogen was raised on cornmeal agar (CMA) for 48 hours to induce sporangial production before being used as inoculum in this trial.

All plots (excluding the uninoculated control treatment 1) were single point inoculated by placed 2 x 1 cm agar discs containing *P. aphanidermatum* into a previously designated inspection port previously prepared in the centre of the troughs, ensuring the discs were sited at the bottom of the port. Inoculation was carried out when the crop reached the wire and when fruit were bulking up on the main stem. Pathogen establishment in the inoculated ports and potential dispersal to other ports in the plot were monitored using a cucumber seedling bioassay and specific serological techniques.

Trial 3:

Because of the infrequent occurrence of *Fusarium* crown and root rot (FCRR) in the North of England it was not considered appropriate to introduce *F. oxysporum* f.sp. *radicis-lycopersici* at HRI Stockbridge House. Therefore, in discussion with HDC representatives, a compromise decision was reached whereby a Fusarium wilt susceptible cultivar (cv. Ailsa Craig) was raised instead and inoculated with a pathogenic isolate of the wilt causing organism *F. oxysporum* f.sp. *lycopersici*.

An isolate of *F. oxysporum* f.sp. *lycopersici*, sourced from Dr J T Fletcher, ADAS Wye, was raised on PDA for 7-10 days to induce sporulation. A spore preparation was made containing ca. 10^5 cells/ml in sterile de-ionised water and 50 ml of this suspension was drenched onto each plant in the designated troughs. All plots (excluding the uninoculated control) were single point inoculated in this way 11 days after planting out. Pathogen establishment in

the inoculated troughs and potential dispersal to other troughs in the plot were monitored using a tomato cv. Ailsa Craig seedling bioassay.

Bait Seedling Preparation and Use

For each trial pathogen-free seedlings were raised in advance in Hassy 308 modules in a 50:50 vermiculite/polystyrene mixture. Plants were maintained in isolation on raised panels and monitored closely prior to use. Cucumber and tomato seedlings were used when the first true leaf became visible after ca. 14 days and ca. 28 days respectively.

At the appropriate growth stage seedlings were removed from the modules and most of the rooting medium shaken off to provide bare root material. Seedlings were inserted into each inspection port (2/port) in each trial and left *in situ* for 2-4 days (5-7 days in Trial 3) ensuring the roots were bathed in hydroponic solution flowing through the bottom of the port.

After this "bait" period seedlings were removed aseptically and placed in individual numbered polythene bags and either returned to the laboratory for further testing, or forwarded to ADAS Leeds for serological analysis.

In addition, the solution collection tanks (pre and post-disinfection) were also "baited" using the same technique. In this case polystyrene rafts were prepared and the bare-rooted seedlings floated in each tank ensuring the roots made contact with the solution. These seedlings remained *in situ* for the same period as those in the growing crop before being returned to the laboratory or ADAS for further testing.

On return to the laboratory seedlings in Trials 1 and 2 were incubated at 20°C for 24 hours in 2 ml sterile pond water and then scored for root discoloration and examined microscopically for the presence of sporangia consistent with those of *P. cryptogea* (Trial 1) or oospores of *P. aphanidermatum* (Trial 2). In Trial 3

seedlings were returned to the laboratory, incubated at 20°C for 2-3 days and subsequently 2-3 mm stem sections excised aseptically and plated out onto PDA. After 4-7 days incubation, isolation plates were checked for the presence of characteristic growth of *F. oxysporum*.

Serological Detection (Trials 1 and 2 only)

Commercially available serological detection kits for *P. cryptogea* and *P. aphanidermatum* were sourced from Agridiagnostic Associates, New Jersey, Hampshire, USA for use in this study. For information on the method of use and application of these kits see Appendix II.

Four types of kit were evaluated:

P. cryptogea

(i) On-site kits (Alert)
(ii) Multiwell plate assays

P. aphanidermatum

(iii) On-site kits (Alert)
(iv) Multiwell plate assays

On-Site Tests

In Trial 1 (tomato) on-site *P. cryptogea* detection kits were used to coincide with "bait" plant removal. Rockwool cores (1 cm diameter) containing tomato root tissue were removed (16 in total) from the inoculated control treatments (T 3-4) and root tissue teased out for use in the serological 'on-site' test. In addition, root tissue from one seedling in each of the baited tanks was used in this test. Procedures for use of the on-site kits were provided by Agridiagnostic Associates. In Trial 2 (cucumber), due to the poor performance of the *P. cryptogea* on-site kits in Year 1 (1992) all tests were conducted on root tissue from "bait" seedlings but using the *P. aphanidermatum* specific kits.

Multi-Well Plate Tests

In Trial 1 four *P. cryptogea* multiwell assay plates were used at most monitoring dates. Two plates were used to monitor dissemination in the inoculated control (T 3-4) using duplicate wells in each plate (1 plate/channel system). One multiwell plate was used (channel system 2 only) for each of the disinfection treatments (T 5-8). Root tissue was excised from each of the "bait" plants (port and tank samples) for use in the multiwell plate assay by ADAS personnel. Known controls were included in all studies as specified by Agridiagnostic Associates.

In Trial 2 four *P. aphanidermatum* multiwell assay plates were used at each monitoring date with duplicate wells in each plate. All treatments in this second trial were monitored using the bait seedlings returned to ADAS Leeds.

Crop Husbandry

The trial crops were grown to a good commercial standard and were maintained in a healthy state (with the exception of the introduced pathogens) and produced adequate yields for representative purposes.

Fungicides were not used for the control of root pathogens though occasional foliar sprays were applied for powdery mildew and botrytis control. These were chosen so as not to interfere with the trial in progress. Pest control was maintained using an IPM approach and chemicals were only resorted to occasionally. All pesticides used were applied at rates as recommended by the manufacturers.

Crop Diary and Cultural Details

Trial 1:

The seed, cv. Liberto, was sown on 14 January and planted out into the cropping house on 16 March 1992. *P. cryptogea* was introduced on 15 May, prior to the first harvest, and pathogen monitoring commenced immediately. Bait seedlings were inserted at 14 day intervals and removed for further testing on 18 May; 1, 15 and 29 June, 13 and 27 July, 24 August, 7 and 21 September, and 9 and 26 October.

Disease assessments were conducted *in situ* on 28 July, 1 and 21 October, and 3 November. The crop finally being terminated on 5 November when detailed assessments of root development and discoloration were undertaken.

Trial 2:

The seed, cv. Corona, was sown on 25 January and planted out into the cropping house on 25 February 1993. *P. aphanidermatum* was introduced on 12 March as the plants reached the cropping wire, and pathogen monitoring commenced immediately.

Bait seedlings were inserted at ca. 21 day intervals and removed for further testing on 26 March, 13 April, 4 and 25 May and 22 June.

Disease assessments were conducted *in situ* on 13, 15 and 30 April, 7 May, 7 and 17 June, and 5 July. The crop finally being terminated on 7 July when detailed assessments of root development and discoloration were undertaken.

Trial 3:

The seed, cv. Ailsa Craig, was sown on 2 July and planted out into the cropping house on 6 August 1993. *F. oxysporum* f.sp. *lycopersici* was introduced on 10 August, four days after planting out and the pathogen monitored subsequently. Bait seedlings, cv. Ailsa Craig, were inserted at approximately monthly intervals and removed for further testing on 31 August, 12 October and 24 November.

Disease assessments were conducted *in situ* on 14 October, 5, 11 and 17 November. The crop finally being terminated on 3 December when detailed assessments of stem infection and root development and discoloration were undertaken.

Disease Assessments

Pathogen Dissemination

Bioassays

At each monitoring date seedlings (cucumber or tomato) were used as "bait" plants in 10 evenly spaced slabs/troughs in each plot (see Bait seedling preparation and use). After the appropriate incubation period the roots of these seedlings were checked for discoloration (+/-) and the presence of the introduced pathogen (+/-) using microscopy. This enabled a calculation of the percentage recovery of the pathogen using the bioassay procedure to be made and this provided a measure of the dissemination of the pathogen under test.

Serological Detection (Trials 1 and 2 only)

Using identical bait seedlings to those used above the presence of the introduced pathogen was determined using pathogen specific serological tests (see Appendix II for full details of test procedure). The presence of the specified pathogen was indicated by a colour change on the multiwell plates and this was recorded on an automated plate reader. Readings over a certain threshold (3 x standard deviation) indicated a positive reaction (ie. confirmation of pathogen presence).

Note:

It was imperative that rooting out from the growing medium into the solution collection channel was avoided in order to accurately determine the method of spread of the introduced pathogen and to interpret the results to judge the performance of the disinfection systems under evaluation. Regular checks of rooting out were made and noted. Where rooting out was detected the root tissue was excised aseptically, in most cases, before the root tissue had grown into the collection channel containing the run-off solution.

In-Crop Disease Development

Whilst emphasis was placed on pathogen detection using seedling bioassays to monitor potential dissemination, the host crop was also used as an 'indicator' of pathogen spread. All three introduced pathogens produce characteristic symptoms in the host crop and these were used to support the results of the bioassay and serological tests.

Wilt assessments: *P. cryptogea* and *F. oxysporum* f.sp. *lycopersici* on tomato and *P. aphanidermatum* on cucumber usually induce a rapid onset of wilting in infected plants and where this symptom occurred it was used as a reliable guide to the rate of dispersal of the introduced pathogens.

At each assessment all plants in the trial area were checked and either recorded as wilted (+) or not (-) or alternatively given a wilt severity score based on the following scale:

0 = No wilt symptoms apparent.

1 = Slight flagging in the 'head' of the plant.

2 = Moderate wilting of many leaves on the plant but likely to recover overnight.

3 = Severe wilting over whole plant, plant unlikely to recover.

In Trial 3, where *F. oxysporum* f.sp. *lycopersici* was inoculated, greater reliance was placed on a visible infection of the stem tissues and this provided valuable information on the potential dissemination of this pathogen.

Crop Vigour

Infection with the specified root pathogens reduced the vigour of the aerial portion of infected plants and where differences became apparent measurements of crop vigour were taken using the following scale:

Crop Vigour Index (1-100)

0 = Very poor vigour, plant dying.

1 = Plant thin and weak, generally very poor growth.

2 = Moderate growth and vigour but reduced compared to other plants.

3 = Vigour excellent, plant appearing very healthy.

In addition, other foliar pathogens occurred in the trials. Their incidence and severity could have been affected by the presence and severity of root disease and therefore random assessments were made at intervals during the trial periods.

Crop Termination Assessments

Ultimately, the roots of each slab were examined in detail when the crop was terminated. Growing slabs were either de-sleeved or removed from the troughs and inverted for assessment. The degree of root development and root discoloration were determined visually using the following 0-3 scales of severity:

Root Development

0 = No root evident at slab base.

1 = <5% surface of slab base covered with root tissue.

2 = 5-25% surface of slab base covered with root tissue.

3 = >25% surface of slab base covered with root tissue.

Root Discoloration

0 = Root severely discoloured, no white root present.

1 = Root moderately discoloured, but some white root evident.

2 = Roots generally white and healthy, but slight evidence of discoloration.

3 = Roots white, extremely healthy.

Where appropriate, core samples were taken aseptically using a sterile cork-borer and returned to the laboratory for confirmation of pathogen presence using microscopy and culture techniques.

Where 0-3 scales were used in assessments an index (0-100) was subsequently calculated using the formula:

$$\frac{1\,(1) + 2\,(2) + 3\,(3)}{\text{No. assessed}} \times \frac{100}{3}$$

In Trial 3, stem sections were taken asceptically from each plant at crop termination and returned to the laboratory for further testing. Small pieces of vascular tissue were excised asceptically and plated out onto PDA. After 5-7 days incubation plates were scored for the presence of fungal colonies consistent with those of *F. oxysporum* f.sp. *lycopersici*.

Yield Records

Fruit were picked at regular intervals throughout the growing season and graded according to normal commercial practice in each trial. Selected data only has been included for comparative purposes in this report. Full details will be retained in the archive at HRI Stockbridge House.

Statistical Analysis

Analysis of the data generated in each year was done with the assistance of HRI Biometrics department. Where necessary the data was transformed prior to analysis (angular, square root and covariate transformation) and is presented in parentheses in the results. Raw data is also presented.

Storage of Data

The raw data from this trials series will be stored for a period of not less than 5 years in the HRI archive at Stockbridge House. Access to the data can be made via the designated archivist.

RESULTS

Trial 1

Seedling Bioassay

Following artificial inoculation on 15 May *P. cryptogea* was detected on a small number of the 'bait' seedlings in the inoculated control treatments but only at the inoculation points (Table 1). *P. cryptogea* was not detected in the uninoculated control or the disinfection treatments at this stage. A significant increase in recovery of the pathogen occurred following baiting on 26 June when *P. cryptogea* was recovered from ca. 70% of 'bait' seedlings in the inoculated control. Pathogen recovery using the seedling bioassay fluctuated but tended to decline towards the end of the trial period. During this period the uninoculated control treatments remained totally free of *P. cryptogea*.

The disinfection treatments using both heat and UV were very effective in minimising dispersal of *P. cryptogea* according to the 'bait' seedling results; recovery levels rarely exceeding 10%, the level at which it was introduced into the monitored parts ie (1 in 10). Only in the UV Vialux treatment (Channel 2) was there any indication of dispersal on 4 September and this was traced back to plants which had rooted out into the collection channel (see Figure 7 in Appendix I).

In the collection, pre and post-treatment tanks (Table 3) *P. cryptogea* was recovered successfully using 'bait' seedlings. The first indication of dissemination was on 12 June in the inoculated control collection tank. By 26 June *P. cryptogea* was detected in the collection tanks of the inoculated control and the UV treatment and in the pre-treatment tanks of both disinfection systems. On no occasion was the pathogen recovered from the uninoculated control collection tank or the two post-treatment disinfection tanks.

Multi-Well Serological Assay

Because of the restricted availability and high cost of the serological tests they were conducted, at selected monitoring dates only, in Channel 2 of both disinfection systems together with the two channel systems in the uninoculated and inoculated control treatments.

Results for the multi-well assay (Table 2) were broadly similar to those of the seedling bioassay though open to some interpretation.

In the inoculated control plots *P. cryptogea* was recovered at the first monitoring date immediately after inoculation and in Channel 1 this was at a level higher than the pathogen was introduced implying dissemination had already occurred. There was considerable inconsistency between wells with some giving a positive reaction, others negative and this made interpretation difficult. As with the seedling bioassay detection of *P. cryptogea* increased dramatically on 26 June though declined thereafter.

Surprisingly, positive confirmation of *P. cryptogea* was also recovered sporadically in the uninoculated control, though only in single wells on the plates.

Pathogen detection in Channel 2 of the Alfa-Laval pasteuriser treatment was below the introduction threshold (10%) using the duplicate well mean except on 26 June when some indication of spread was gained.

In Channel 2 of the Priva UV 'Vialux' treatment pathogen detection was below the inoculation threshold (using the duplicate well mean). In both disinfection treatments there was a general decline in detection once the peak recovery had been reached on 26 June.

In the collection, pre and post-treatment tanks *P. cryptogea* was detected successfully using the multi-well assay. Positive confirmation was gained in the collection tank of the inoculated control immediately after inoculation on 15 May and also in the pre-treatment tank of the UV treatment. However, positive confirmations were also made in the collection tanks of the uninoculated control on 26 June, 6 and 23 October. Some positive reactions were also recorded (single wells only) in the post-treatment tanks of both disinfection system. This raises some doubt as to the validity of this serological test.

On-Site Serological Assays

As with the multi-well assays testing was somewhat restricted with the on-site kits due to their limited availability and high cost. Tests were confined to the inoculated control and the 'bait' seedlings in the collection tanks (Table 5).

Very few positive results were achieved in the on-site tests. The first positive results were recorded on 12 June from the inoculated control plots. The earliest detection in the collection tanks was on 24 July in the UV treatment and this was some five weeks after the first confirmation using microscopy of the same bait seedlings. No confirmation of *P. cryptogea* was obtained in either the pre or post-treatment tanks using this technique during the study.

Disease Aetiology

No symptoms of *P. cryptogea* were apparent in the aerial canopy until early July, some two months after it was first detected on seedlings used to 'bait' the root zone. By early July occasional plants at the inoculation points in each plot were observed to wilt on hot, sunny days.

By late July when there were sufficient symptoms for an assessment to be undertaken 28% plants in the inoculated control were wilted (Table 6). By comparison <1% were affected in the uninoculated control and only 2-3% in the disinfection treatments (the latter being a reflection of plants showing symptoms at the inoculation points).

As the season progressed the incidence of wilting increased in the inoculated control until by early October 80% plants exhibited wilt symptoms on hot days with high solar radiation (see Figure 8 in Appendix I). In comparison, the incidence of wilting in the uninoculated control remained negligible and the crop appeared totally disease-free.

Following disinfection by both heat and UV a low incidence of wilting was maintained, largely reflecting the occurrence of disease at the inoculation points. Occasional foci of infected plants were found towards the end of the trial particularly in Channel 2 of the pasteuriser treatment. Where affected plants occurred it invariably co-coincided with plants which had rooted out into the collection channel and therefore picked up infection prior to disinfection.

At crop termination, a final assessment of wilting was made and the incidence of dead plants was also recorded (Table 7). A moderate-high incidence of wilting remained in the inoculated plots and this was markedly reduced in the disinfected plots. Interestingly, the incidence of wilting following disinfection by heat treatment was higher than where UV disinfection had been used and this was consistent across both channel systems.

It was not possible to accurately record the incidence of dead plants in the inoculated control at termination due to the severity of wilt symptoms at this time. However, very few losses occurred in the uninoculated control (mean of 4.4%) as compared with a mean of 17.5% for the pasteuriser and a mean of 10.8% for the UV treatment. The increase in dead plants compared to the control was largely due to disease at the inoculation points in the experimental plots.

At the end of the trial all the roots were assessed for both development and discoloration (Table 7).

The uninoculated plots gave a mean index of 94.7 for root development whereas following dissemination of *P. cryptogea* in the inoculated control this was reduced dramatically to a mean index of 35.6, a clear measure of the severe effect *P. cryptogea* had on the root system of the crop. In contrast, following disinfection the root development index was retained at a high level with both the pasteuriser (I = 92.8) and the UV system (I = 91.6).

The control slabs exhibited some root discoloration at crop termination and this was reflected in a mean index of 34.5. However, this was increased significantly in the inoculated control where the roots of all slabs were severely discoloured (I = 99.9). Again, where heat or UV disinfection had been deployed root discoloration was minimised (Table 7).

Interestingly, black dot (*Colletotrichum coccodes*) occurred naturally in the root zone of this trial and at termination over 80% of the slabs in the uninoculated control were affected and this may account for some or all of the root discoloration observed in this treatment. In the *P. cryptogea* inoculated plots the incidence of *C. coccodes* was much reduced in a visual assessment (36% slabs affected). This may have been due to (a) increased competition for infection sites or (b) masking of symptoms of *C. coccodes* due to the severity of the *P. cryptogea* infection on the roots. High levels were also detected in both the disinfection treatments (Pasteuriser - 87.9%; UV 67.1%) and this suggests that disinfection is ineffective in preventing dissemination of *C. coccodes*. However, the method of introduction and subsequent dispersal of *C. coccodes* has not been ascertained in this trial and therefore the result should be interpreted with caution.

Yield

The mean yield in the inoculated varied slightly between the two channel systems, with Channel 1 slightly higher than Channel 2, providing a mean total yield of 38.2 kg/m^2. Surprisingly, given that *P. cryptogea* was disseminated widely through the inoculated control plots, by late June the mean total yield for these plots was 36.3 kg/m^2. **This represents a yield reduction of only 5% over the duration of the trial.**

Disinfection by both heat and UV increased the total yield above that of the uninoculated control (Pasteuriser, 39.1 kg/m^2, UV, 40.1 kg/m^2). This is somewhat surprising as at least three plants per plot (at the inoculation point) were infected by *P. cryptogea*.

Nutrient Status

The effect of disinfection by pasteurisation and UV are presented in Table 24, Appendix IV following analysis of a series of 10 samples taken during the period 4 August to 7 October 1992.

Treatment of the hydroponic solution with Alfa-Laval pasteuriser added approximately 18% copper to the nutrient solution. This may have been due to the copper content of the heat exchange plates or other ancillary equipment in the design of this disinfection rig. The Priva UV 'Vialux' unit removed approximately 40% iron through oxidation and coating of the quartz UV tube. The modifications to the nutrient status are not considered to be of significant commercial importance as the solution being treated comprises 30-40% run-off solution of the total nutrient solution requirements. The addition of a significant quantity of fresh nutrient solution at each watering is likely to negate any significant effect. Clearly, however, the deposition of iron on the quartz UV tube is important as it will potentially cause a decline in performance of the UV equipment. In the case of the Priva 'Vialux' this is accommodated by the installation of an automatic lamp wiping facility which prevents a build up of iron on the quartz tube and UV transmissions is not affected significantly.

Table 1: Percentage recovery of P. cryptogea using microscopy on 'bait' tomato seedlings at intervals following artificial introduction at a single focus in each plot.

Treatments	15/5	29/5	12/6	26/6	10/7	24/7	6/8	21/8	4/9	18/9	6/10	23/10
Uninoculated control (T1)												
Channel 1	0.0 (-)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	-	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Channel 2	0.0 (-)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	-	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Mean	**0.0 (-)**	**0.0 (0.0)**	**0.0 (0.0)**	**0.0 (0.0)**	**0.0 (0.0)**	**0.0 (0.0)**	-	**0.0 (0.0)**	**0.0 (0.0)**	**0.0 (0.0)**	**0.0 (0.0)**	**0.0 (0.0)**
Inoculated Control (T2)												
Channel 1	2.5 (-)	0.0 (0.0)	5.0 (9.2)	70.0 (58.3)	7.25 (58.6)	35.0 (36.1)	-	70.0 (56.3)	50.0 (45.0)	40.0 (38.4)	37.5 (36.9)	25.0 (26.3)
Channel 2	0.0 (-)	5.0 (9.2)	5.0 (6.6)	55.5 (47.9)	65.0 (54.0)	30.0 (32.3)	-	55.0 (47.6)	65.0 (55.3)	27.5 (30.8)	35.0 (34.5)	40.0 (38.9)
Mean	**1.3 (-)**	**2.5 (4.6)**	**5.0 (7.9)**	**62.5 (53.1)**	**68.8 (56.3)**	**32.5 (34.2)**	-	**62.0 (51.9)**	**57.0 (50.2)**	**33.8 (34.6)**	**36.3 (36.2)**	**32.5 (32.6)**
Disinfection with Heat (Alfa Laval Pasteuriser) (T3)												
Channel 1	0.0 (-)	2.5 (4.6)	2.5 (4.6)	7.5 (11.3)	7.5 (11.3)	7.5 (11.3)	-	5.0 (9.2)	5.0 (9.2)	2.5 (4.6)	7.5 (11.3)	0.0 (0.0)
Channel 2	0.0 (-)	5.0 (9.2)	5.0 (9.2)	2.5 (4.6)	10.0 (12.9)	7.5 (11.3)	-	7.5 (8.3)	10.0 (12.7)	7.5 (11.3)	7.5 (11.3)	2.5 (4.6)
Mean	**0.0 (-)**	**3.8 (6.9)**	**3.8 (6.9)**	**5.0 (7.9)**	**8.8 (12.1)**	**7.5 (11.3)**	-	**6.3 (8.8)**	**7.5 (10.9)**	**5.0 (7.9)**	**7.5 (11.3)**	**1.3 (2.3)**
Disinfection with UV (Priva Vialux') (T4)												
Channel 1	0.0 (-)	5.0 (9.2)	5.0 (9.2)	7.5 (13.8)	10.0 (15.9)	10.0 (15.9)	-	5.0 (9.2)	7.5 (13.8)	2.5 (4.6)	10.0 (15.9)	7.5 (13.9)
Channel 2	0.0 (-)	0.0 (0.0)	2.5 (4.6)	5.0 (9.2)	7.5 (13.8)	5.0 (9.2)	-	5.0 (9.2)	2.5 (4.6)	2.5 (4.6)	7.5 (13.8)	0.0 (0.0)
Mean	**0.0 (-)**	**2.5 (4.6)**	**3.8 (6.9)**	**6.3 (11.5)**	**8.8 (14.8)**	**7.5 (12.5)**	-	**5.0 (9.2)**	**5.0 (9.2)**	**2.5 (4.6)**	**8.8 (14.8)**	**3.8 (6.9)**
Mean of Channel 1	0.63 (-)	1.9 (3.7)	3.1 (6.1)	21.3 (22.2)	22.5 (22.9)	13.1 (16.8)		20.0 (19.9)	15.6 (18.1)	11.3 (12.7)	13.8 (17.1)	8.1 (10.7)
Mean of Channel 2	0.0 (-)	2.5 (4.9)	3.1 (5.5)	15.6 (16.5)	20.6 (21.5)	10.6 (14.1)		16.9 (17.4)	22.5 (19.4)	9.4 (12.4)	12.6 (16.1)	10.6 (11.6)
Treatment Significance	(-)	(NS)	(NS)	(***)	(***)	(***)		(***)	(***)	(***)	(***)	(***)
LSD (5%) for comparing mean of T1 with T2-T4	(-)	(-)	(-)	(11.53)	(11.32)	(11.58)		(14.74)	(11.71)	(11.40)	(13.11)	(10.40)
LSD (5%) for comparing mean of T2, T3 and T4	(-)	(-)	(-)	(10.68)	(10.48)	(10.72)		(13.65)	(10.84)	(10.55)	(12.13)	(9.63)
Channel Type Significance	(-)	(NS)	(NS)	(NS)	(NS)	(NS)		(NS)	(NS)	(NS)	(NS)	(NS)
LSD (5%) for comparing means of channel types	(-)	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)	(-)	(-)

NB. Confirmation based on presence of sporangia consistent with those of P. cryptogea as observed under the microscope.
- No test carried out on this date.
Angular transformation of data conducted prior to analysis. Transformed data is presented in parentheses.

Table 2: Percentage recovery of P. cryptogea using a serological test (Multi-Well Plate Assay - duplicate well) on 'bait' tomato seedlings at intervals following artificial introduction at a single 'focus in each plot.

Treatments	15/5	29/5	12/6	26/6	10/7	24/7	6/8	21/8	4/9	18/9	6/10	23/10
	Date											
Uninoculated control (T1)												
Channel 1*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (1.9)	-	0.0 (1.3)	-	-	-	-	0.0 (0.0)	0.0 (0.0)
Channel 2*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (-1.9)	-	0.0 (-1.3)	-	-	-	-	0.0 (0.0)	0.0 (0.0)
Mean	**0.0 (0.0)**	**0.0 (0.0)**	**0.0 (0.0)**	**0.0 (0.0)**	-	**0.0 (0.0)**	-	-	-	-	**0.0 (0.0)**	**0.0 (0.0)**
Inoculated Control (T2)												
Channel 1	15.0 (22.5)	0.0 (0.0)	10.5 (15.9)	75.0 (61.2)	-	22.5 (29.4)	-	-	-	-	30.0 (32.9)	27.5 (27.7)
Channel 2	2.5 (4.6)	5.0 (9.2)	12.5 (20.5)	62.5 (52.6)	-	12.5 (20.5)	-	-	-	-	40.0 (38.9)	42.5 (40.6)
Mean	**8.8 (13.6)**	**2.5 (4.6)**	**11.25 (18.2)**	**68.8 (56.9)**	-	**17.5 (25.0)**	-	-	-	-	**35.0 (35.9)**	**35.0 (34.2)**
Disinfection with Heat (Alfa Laval Pasteuriser) (T3)												
Channel 1	-	-	-	-	-	-	-	-	-	-	- -	- -
Channel 2	0.0 (0.0)	5.0 (9.2)	7.5 (13.8)	12.5 (17.9)	-	10.0 (15.9)	-	-	-	-	# (#)	0.0 (0.0)
Mean												
Disinfection with UV (Priva Vialux') (T4)												
Channel 1	-	-	-	-	-		-	-	-	-	- -	- -
Channel 2	10.0 (13.3)	7.5 (11.3)	5.0 (9.2)	2.5 (4.6)	-	5.0 (9.2)	-	-	-	-	7.5 (13.8)	0.0 (0.0)
Mean												
Mean of Channel 1	7.0 (12.9)	0.0 (0.0)	5.0 (9.1)	37.5 (35.8)	-	12.5 (17.3)	-	-	-	-	15.0 (18.8)	13.8 (15.8)
Mean of Channel 2	3.1 (4.8)	4.4 (7.9)	6.3 (11.6)	19.4 (19.6)	-	6.9 (11.9)	-	-	-	-	15.8 (19.2)	10.6 (10.8)
Treatment Significance	(**)	(NS)	(*)	(***)		(***)					(***)	(***)
LSD (5%) for comparing mean of T1 with T2-T4	(12.97)	(-)	(12.95)	(12.00)	-	(11.05)	-	-	-	-	(12.76)	(14.94)
LSD (5%) for comparing mean of T2, T3 and T4	(12.01)	(-)	(11.99)	(11.11)	-	(10.23)	-	-	-	-	(11.82)	(13.83)
Channel Type Significance	(*)	(NS)	(NS)	(***)	-	(NS)	-	-	-	-	(NS)	(NS)
LSD (5%) for comparing mean of Channel types	(7.77)	(-)	(-)	(7.21)		(-)	-	-	-	-	(7.65)	(8.95)

* Random test only - Not tested on this date # Faulty plate (no dessicant present)

Note: Positive confirmation based only on duplicate wells above normal threshold (X + 3 x standard deviation).

Angular transformation of data conducted prior to analysis. Transformed data is presented in parentheses.

Table 2a: Percentage recovery of P. cryptogea using a serological test (Multi-Well Plate Assay - single well) on 'bait' tomato seedlings at intervals following artificial introduction at a single 'focus in each plot.

Treatments	Date: 15/5	29/5	12/6	26/6	10/7	24/7	6/8	21/8	4/9	18/9	6/10	23/10
Uninoculated control (T1)												
Channel 1*	0.0 (0.0)	0.0 (0.0)	2.5 (6.1)	0.0 (1.9)	-	0.0 (1.6)	-	-	-	-	0.0 (0.0)	0.0 (0.0)
Channel 2*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (-1.9)	-	2.5 (4.5)	-	-	-	-	0.0 (0.0)	0.0 (0.0)
Mean	**0.0 (0.0)**	**0.0 (0.0)**	**1.3 (3.1)**	**0.0 (0.0)**	-	**1.3 (3.1)**	-	-	-	-	**0.0 (0.0)**	**0.0 (0.0)**
Inoculated Control (T2)												
Channel 1	42.5 (40.6)	7.5 (11.3)	12.5 (2.05)	80.0 (64.3)	-	35.0 (3.62)	-	-	-	-	47.5 (43.3)	30.0 (29.4)
Channel 2	2.5 (4.6)	5.0 (9.2)	22.5 (27.7)	70.0 (57.1)	-	15.0 (22.5)	-	-	-	-	45.0 (41.8)	47.5 (43.6)
Mean	**22.5 (22.6)**	**6.3 (10.3)**	**17.5 (24.1)**	**75.0 (60.7)**	-	**25.0 (29.4)**	-	-	-	-	**46.3 (42.6)**	**38.8 (36.5)**
Disinfection with Heat (Alfa Laval Pasteuriser) (T3)												
Channel 1	-	-	-	-	-	-	-	-	-	-	-	-
Channel 2	0.0 (0.0)	7.5 (11.3)	12.5 (20.5)	32.5 (30.9)	-	10.0 (15.9)	-	-	-	-	# (#)	0.0 (0.0)
Mean												
Disinfection with UV (Priva Vialux') (T4)												
Channel 1	-	-	-	-	-		-	-	-	-	-	-
Channel 2	22.5 (27.3)	22.5 (27.3)	5.0 (9.2)	5.0 (9.2)	-	10.0 (13.3)	-	-	-	-	7.5 (13.8)	0.0 (0.0)
Mean												
Mean of Channel 1	21.3 (23.2)	3.95 (6.4)	7.5 (14.3)	40.0 (37.6)	-	17.5 (21.4)	-	-	-	-	23.75 (24.8)	15.0 (16.8)
Mean of Channel 2	6.25 (8.5)	8.99 (12.7)	10.0 (15.3)	26.9 (25.5)	-	9.4 (14.7)	-	-	-	-	17.5 (20.2)	11.9 (11.6)
Treatment Significance	(***)	(*)	(***)	(***)	-	(***)	-	-	-	-	(***)	(***)
LSD (5%) for comparing mean of T1 with T2-T4	(11.09)	(16.95)	(5.69)	(15.52)	-	(12.70)	-	-	-	-	(17.12)	(15.79)
LSD (5%) for comparing mean of T2, T3 and T4	(10.27)	(15.69)	(5.26)	(14.37)	-	(11.75)	-	-	-	-	(15.85)	(14.62)
Channel Type Significance	(***)	(NS)	(NS)	(*)	-	(NS)	-	-	-	-	(NS)	(NS)
LSD (5%) for comparing mean of Channel types	(6.65)	(-)	(-)	(9.32)	-	(-)	-	-	-	-	(-)	(-)

* Random tests only - Not tested on this date # Faulty plates (no dessicant present)

Note: Positive confirmation based only on single well above normal threshold (X + 3 x standard deviation).

Angular transformation of data conducted prior to analysis. Transformed data is presented in parentheses.

Table 3: Recovery (+/-) of *P. cryptogea* from nutrient solution in the collection, pre and post-treatment tanks using microscopy on 'bait' seedlings following artificial introduction at a single focus in each plot.

	Date											
Treatment	15/5	29/5	12/6	26/6	10/7	24/7	6/8	21/8	4/9	18/9	6/10	23/10
Collection Tanks												
1. Uninoculated	-	-	-	-	-	-	*	-	-	-	-	-
2. Inoculated	-	-	+	+	+	+	*	+	+	+	+	-
3. Pasteuriser	-	-	-	-	+	+	*	+	+	+	+	+
4. UV	-	-	-	+	+	+	*	+	+	?	+	+
Pre-Treatment Tanks												
5. Pasteuriser	-	-	-	+	+	+	*	-	-	+	+	-
6. UV	-	-	-	+	+	+	*	-	-	+	-	+
Post-Treatment Tanks												
7. Pasteuriser	-	-	-	-	-	-	*	-	-	-	-	-
8. UV	-	-	-	-	-	-	*	-	-	-	-	-

+ Positive confirmation of *P. cryptogea* based on visual diagnosis of sporangia conforming to *P. cryptogea*, using microscopy.

- No sporangia of *P. cryptogea* detected using microscopy.

* Not tested on that date.

? Doubtful confirmation based on morphological characteristics of sporangia.

Table 4: **Recovery (+/-) of *P. cryptogea* from nutrient solution collected in the collection and pre and post-treatment tanks using serological multi-well plates following artificial introduction at a single focus in each plot.**

Treatment	Date											
	15/5	29/5	12/6	26/6	10/7	24/7	6/8	21/8	4/9	18/9	6/10	23/10
Collection Tanks												
1. Uninoculated	-	-	-	+	*	-	-	-	-	-	+	+
2. Inoculated	+	-	+	+	*	+	-	-	-	-	+	-
3. Pasteuriser	-	-	-	+	*	+	-	-	-	-	+	-
4. UV	-	-	+#	+	*	+	-	-	-	-	+	+
Pre-Treatment Tanks												
5. Pasteuriser	-	-	-	+	-	-	-	-	-	-	-	+
6. UV	+	-	+	+	-	+	-	-	-	-	-	-
Post-Treatment Tanks												
7. Pasteuriser	-	-	-	+#	-	-	-	-	-	-	-	-
8. UV	-	-	-	-	-	-	-	-	-	-	-	+#

+ Confirmation based on duplicate wells above normal threshold (x + 3 x sd).

- No confirmation of *P. cryptogea*.

Positive reaction in one well only.

* Not tested on this date.

Table 5: Percentage recovery of *P. cryptogea* using a serological test (Alert On-Site tests) *in situ* at intervals following artificial introduction at a single focus in each plot.

	Date											
Treatments	15/5	29/5	12/6	26/6	10/7	24/7	6/8	21/8	4/9	18/9	6/10	23/10
Uninoculated treatments	*	*	*	*	*	*	*	*	*	*	*	*
Inoculated												
Channel 1	*	*	*	*	*	*	*	*	*	*	*	*
Channel 2	0.0	0.0	16.7	25.0	*	16.7	*	*	*	50.0	8.3	*
Pasteuriser	*	*	*	*	*	*	*	*	*	*	*	-
UV	*	*	*	*	*	*	*	*	*	*	*	-
Collection Tanks												
1. Uninoculated	-	-	-	-	*	?	*	*	*	-	?	*
2. Inoculated	-	-	-	-	*	?	*	*	*	-	+	*
3. Pasteuriser	-	-	-	-	*	-	*	*	*	-	+	*
4. UV	-	-	-	-	*	+	*	*	*	-	-	*
Pre-Treatment Tanks												
5. Pasteuriser	-	-	-	-	*	-	*	*	*	-	-	*
6. UV	-	-	-	-	*	-	*	*	*	-	-	*
Post-Treatment Tanks												
7. Pasteuriser	-	-	-	-	*	-	*	*	*	-	-	*
8. UV	-	-	-	-	*	-	*	*	*	-	-	*

\+ Positive confirmation of *P. cryptogea* based on colour change in Alert test.
\- No confirmation of *P. cryptogea* ie no colour change.
? Unclear result
* Not tested on this date.

Note. On-site monitoring using Alert kits was only conducted in the inoculated control (Channel System 2) plots. In each of the four replicates three slabs were designated, one being the inoculation point. In the tanks roots from a 'bait' seedling were used. A core sample, include root tissues was taken from each slab for testing.

Table 6: Assessments of wilting and dead plants at intervals during the trial period following 'artificial inoculation with P. cryptogea at a single focus in each plot.

Treatment	% Plants Wilting 28 July#		1 October#		21 October	% Dead Plants+ 21 October
Uninoculated control (T1)						
Channel 1	1.1	(0.49)	0.0	(-0.03)	1.3	2.7
Channel 2	0.06	(0.55)	0.0	(0.03)	2.4	1.7
Mean	**0.8**	**(0.52)**	**0.0**	**(0.0)**	**1.8**	**2.2**
Inoculated Control (T2)						
Channel 1	36.3	(5.90)	84.6	(9.19)	84.5	7.5
Channel 2	16.2	(3.95)	76.7	(8.74)	77.8	17.8
Mean	**26.2**	**(4.92)**	**80.7**	**(8.97)**	**81.1**	**12.7**
Disinfection with Heat (Alfa Laval Pasteuriser) (T3)						
Channel 1	3.7	(1.90)	3.8	(2.13)	5.5	6.5
Channel 2	4.2	(1.99)	4.6	(1.88)	13.3	7.5
Mean	**4.0**	**(1.94)**	**4.2**	**(2.01)**	**9.4**	**7.0**
Disinfection with UV (Priva Vialux') (T4)						
Channel 1	3.3	(1.79)	3.3	(1.79)	9.2	2.3
Channel 2	2.5	(1.56)	3.8	(1.83)	5.5	5.3
Mean	**2.9**	**(1.68)**	**3.5**	**(1.81)**	**7.4**	**3.8**
Mean of Channel 1	11.1	(2.66)	22.9	(3.49)	26.7	4.8
Mean of Channel 2	5.9	(2.11)	21.3	(3.33)	26.2	8.0
Significance		(***)		(***)	***	-
LSD (5%) for comparing mean of T1 with T2-T4		(0.89)		(0.52)	5.14	-
Significance		(***)		(***)	***	-
LSD (5%) for comparing mean of T2, T3 and T4		(0.82)		(0.48)	4.76	-
Significance		(NS)		(NS)	NS	-
LSD (5%) for comparing mean of channel types		(-)		(-)	(-)	-

Square root transformation of data prior to analysis. Transformed data in parentheses.
+ No analysis conducted.

Table 7: Disease assessments at crop termination on 5 November 1992.

Treatment	Wilt Index (0-100)	% Dead Plants+	Root Development Index (0-100)	Root Discoloration Index (0-100)	% Slabs with Colletotrichum Infection+
Uninoculated control (T1)					
Channel 1	4.3	6.6	93.8	34.7	84.4
Channel 2	1.8	2.2	95.1	34.1	81.1
Mean	**3.1**	**4.4**	**94.5**	**34.5**	**82.7**
Inoculated Control (T2)					
Channel 1	62.9	*	35.2	99.7	26.6
Channel 2	71.9	*	35.8	100.0	45.0
Mean	**67.4**	*	**35.5**	**99.9**	**35.8**
Disinfection with Heat (Alfa Laval Pasteuriser) (T3)					
Channel 1	12.2	15.8	90.0	40.5	86.6
Channel 2	14.3	19.2	90.5	42.5	89.2
Mean	**13.2**	**17.5**	**90.2**	**41.5**	**87.9**
Disinfection with UV (Priva Vialux') (T4)					
Channel 1	6.4	8.3	92.2	41.3	62.5
Channel 2	8.4	13.3	91.1	41.1	71.6
Mean	**7.4**	**10.8**	**91.6**	**42.2**	**67.1**
Mean of Channel 1	22.6	7.7	76.7	55.4	65.0
Mean of Channel 2	25.6	8.9	77.0	55.8	71.7
Significance	***	-	***	***	-
LSD (5%) for comparing mean of T1 with T2-T4	6.49	-	4.34	3.22	-
Significance	***	-	***	***	-
LSD (5%) for comparing mean of T2, T3 and T4	6.01	-	4.02	2.98	-
Significance	NS	-	NS	NS	-
LSD (5%) for comparing mean of channel types	-	-	-	-	-

+ No analysis conducted
* Not assessed due to overall severity of wilt symptoms in these plots at the time the assessment was conducted.

Table 8: Yield data for the tomato trial inoculated with *P. cryptogea* in House 5 (1992).

Treatment	Total Yield (kg/m^2)	% Class I of Total Yield	% Waste of Total Yield
Uninoculated Control			
Channel 1	38.56	67.18	1.16
Channel 2	37.88	66.79	1.19
Mean	**38.22**	**66.99**	**1.17**
Inoculated Control			
Channel 1	34.57	69.28	1.45
Channel 2	37.94	67.98	1.37
Mean	**36.26**	**68.63**	**1.41**
Disinfection with Heat (Alfa-Laval pasteuriser)			
Channel 1	39.22	67.19	1.42
Channel 2	39.02	67.14	1.64
Mean	**39.12**	**67.17**	**1.53**
Disinfection with UV (Priva 'Vialux')			
Channel 1	39.91	65.84	1.44
Channel 2	40.29	65.31	1.41
Mean	**40.10**	**65.58**	**1.43**
Significance	**	*	*
SED (19 df)	0.63	1.39	0.16
LSD (5%)	1.31	2.90	0.33

Trial 2 (January - July 1993)

Seedling Bioassay

Following artificial inoculation on 12 March *P. aphanidermatum* was immediately detected in a small number of the 'bait' seedlings in all the inoculated treatments (T2-T6). In all cases, the detection coincided with the pathogen inoculation points in each plot (Table 9).

A significant increase in recovery of *P. aphanidermatum* occurred following baiting on 8 April when the pathogen was recovered from 53% of 'bait' seedlings in the inoculated control. Detection levels in the disinfection treatments remained fairly constant and below the 10% threshold level at which it was introduced, ie one inoculated port out of 10 monitored. *P. aphanidermatum* was not detected in the uninoculated control treatment on 8 April. By the next monitoring on 30 April the level of recovery in the inoculated control had risen to 80% indicating widespread dissemination of *P. aphanidermatum* in the absence of any disinfection treatment. Somewhat disconcertingly, *P. aphanidermatum* was also recovered from 37% of the 'bait' plants in the un inoculated control and this provided a strong indication that some form of aerial transmission of the pathogen had occurred in the trial to infest the solution. Re-assuringly, however, pathogen recovery in the disinfected plots remained low. This trend continued through to crop termination when 93% plants were estimated to be infected with *P. aphanidermatum* in the inoculated control; 80% in the uninoculated control but only 0-5% in the various disinfection treatments.

In the collection, pre and post-treatment tanks *P. aphanidermatum* was not detected in the initial batch of 'bait' seedlings inserted into the tanks on 12 March (Table 11).

By 8 April, when the second batch of seedlings were examined the pathogen was detected in all the collection tanks and two of the pre-treatment tanks (pasteuriser and UV). More importantly, oospores consistent with those of *P. aphanidermatum* were found in the root tissues of seedlings used to bait the post-treatment tanks of the UV and the ozone treatments. Positive confirmations continued to be made in the collection and pre-treatment tanks at subsequent monitoring intervals on 30 April, 21 May and 18 June. Even though positive detection in two of the post-treatment tanks had been recovered on 8 April this was not confirmed at the next monitoring on 30 April. However, positive results continued to be gained for the UV and ozone post-treatment tanks on 21 May and with the UV and micro-filtration post-treatment tanks on 18 June (Table 11).

Multi-Well Serological Assay

Following pathogen inoculation on 12 March, the first serological assay of the first batch of 'bait' seedlings detected *P. aphanidermatum* at all the inoculation points (Table 10). Positive confirmation of the pathogen was also gained at other (uninoculated) locations in the inoculated control (T2), the Priva UV 'Vialux' (T4), the Ozotech ozoniser (T5) and in the Memcor micro-filtration unit (T6). At the second assay on 8 April the pathogen was detected at a high level in the inoculated control (60%) but also at a low level (three slabs) in the uninoculated control. Confirmation of the pathogen in the disinfected plots was primarily at the inoculation points, but with occasional recovery in isolated uninoculated locations in T3 (pasteuriser) and T6 (micro-filtration). By the third serological assay, *P. aphanidermatum* was recovered at 100% of monitoring points in the inoculated control and at 70% of monitoring points in the uninoculated. This latter figure also rose to 100% at later monitoring dates (Table 10).

Pathogen detection in the disinfection treatments using serology increased at the third assay on 30 April and according to this detection technique *P. aphanidermatum* had disseminated to affect 15%, 20%, 35% and 45% of plants in the pasteuriser, UV, ozone and micro-filtration treatments respectively. However, in subsequent assays, the incidence of positive confirmation using serology declined.

In the nutrient solution collection tanks and the pre and post-treatment tanks (Table 12) a more consistent result was achieved though even this did not 'mirror' the results of the microscopy 'bioassay'.

P. aphanidermatum was first detected at the second assay on 8 April in most collection tanks (excluding the uninoculated) but not the pre or post-treatment tanks. However, by the third assay on 31 April it was also detected in three of the four pre-treatment tanks, but in none of the post-treatment tanks. By 21 May the pathogen was also detected in three of the four post-treatment tanks (pasteuriser, ozoniser and micro-filtration tanks) but surprisingly it was not detected in any of these tanks at the final assay on 18 June (Table 12).

<u>On-Site Serological Assay</u>

No on-Site testing was done in this trial using the Alert kits as they were unavailable from the manufacturers.

<u>Disease Aetiology</u>

Characteristic symptoms of infection by *P. aphanidermatum* first became apparent on 5 April, 3-4 weeks after inoculation. Occasional plants in inoculated troughs were observed to wilt when under stress during periods of high temperature/solar radiation. A detailed assessment was conducted on 13 April during overcast weather and this indicated a higher incidence of wilting in the inoculated control than any other treatment (Table 13). This assessment was repeated on 15 April due to a change in the weather from dull and overcast to bright and sunny, when a significant increase in wilting was recorded in the inoculated control.

A small number of plants at the inoculation point were also wilting in some of the disinfection treatments. More disconcerting was the occurrence of occasional plants wilting in the uninoculated control and this was the first visual indication of lateral (aerial?) movement of *P. aphanidermatum* in the cropping house. By the 30 April the incidence and severity of wilt symptoms had increased to similar levels in both uninoculated and inoculated controls. In contrast, wilt symptoms were largely confined to the inoculation points in the disinfected plots (see Figure 9 in Appendix I). A final wilt assessment conducted again during high temperature/solar radiation conditions on 7 June showed a marked reduction in wilt symptoms in the control plots (Table 13). Plants in the disinfection treatments remained largely healthy through to crop termination in early July.

Some foliar diseases, primarily grey mould (*Botrytis cinerea*), white rot (*Sclerotinia sclerotiorum*) and *Penicillium oxalicum* occurred in the aerial canopy of this crop and were assessed at intervals on the main stem (Tables 14 and 15). Grey mould was the only significant pathogen and was most severe in the uninoculated (but subsequently infected) control. Stem lesions caused by *B. cinerea* were largely superficial and only rarely were plants killed by this disease in the trial. The remaining pathogens remained at low-insignificant levels throughout and were judged to have little or no effect in the crop.

A plant vigour assessment conducted at crop termination on 5 July demonstrated that the plants had been significantly weakened by *P. aphanidermatum* root infection in both inoculated and uninoculated controls. By contrast, vigour remained high in all disinfection treatments and this provided the ultimate test on the performance of the equipment.

Assessments of root development and root discoloration were conducted at crop termination (Table 16). Root development was relatively poor in both control treatments where *P. aphanidermatum* had been disseminated widely. Where disinfection systems had been deployed root development was much improved and this was particularly marked in Treatment 6 (micro-filtration).

Roots were very discoloured in the control treatment, the more so in the inoculated (DI = 95.6) as compared with the uninoculated control (DI = 61.1). Root discoloration was significantly reduced in all disinfection treatments but particularly in the micro-filtration treatment (DI = 8.3). In this treatment root vigour was exceptional and little discoloration was apparent (see Figure 10 in Appendix I).

Yield

Unfortunately due to the lateral (aerial?) dispersal of *P. aphanidermatum* into the uninoculated control no base-line yield for comparison with inoculated treatments is available. Yield, in both treatments where *P. aphanidermatum* was dispersed, and caused widespread wilt symptoms, was low (Table 17), with the uninoculated yielding less than the equivalent inoculated treatment. This is somewhat surprising in view of the considerable delay in pathogen establishment in Treatment 1.

In comparison, the yield was increased significantly following disinfection with heat, UV, ozone and micro-filtration and this adds further support to the value of disinfection systems for prevention of pathogen dissemination in recirculation systems.

The micro-filtration system provided the highest yield in this trial, though only marginally as compared with the ozone and UV systems and this represented a yield increase as compared with the inoculated control of 22%.

Nutrient Status

The effect of disinfection by pasteurisation, UV, ozone and micro--filtration on the nutrient status of the hydroponic solution are presented in Table 25, Appendix IV, following analysis of a series of four samples taken during the period 21 April and 30 June.

Treatment of the Brinkman 'Drainheater' pasteuriser had relatively little effect on the nutrient status with only small reductions in K, Ca and Mg. An increase in phosphorus may be accounted for by the automatic use of phosphoric acid to prevent limescale on the heat exchange plates.

Following UV treatment there appeared to be significant reductions in a number of nutrients, primarily Fe, Ca, Mg and Mn. This could be due to oxidation and deposition on the quartz UV lamp, as described in the results for the 1992 tomato trial. A satisfactory explanation for the marked increase in HCO_3 following UV treatment has not, as yet, been forthcoming.

Treatment with ozone caused a reduction in the nutrient status of certain elements in solution. Fe, Mn and Zn appeared to be oxidised and removed from solution to the greatest extent.

Use of the passive micro-filtration had relatively little effect on the nutrients in solution. Several elements declined in concentration following disinfection, notably Fe, P, Mg and S. Whether this effect was due to continued microbial activity in solution is open to some speculation.

Table 9: Percentage recovery of *P. aphanidermatum* using microscopy on 'bait' cucumber seedlings at intervals following artificial introduction at a single focus in each plot.

Treatments	Date 12/3	8/4	30/4	21/5	18/6
Uninoculated control (T1)	0.0 (0.0)	0.0 (0.0)	36.7 (36.1)	63.3 (28.1)	0.0 (64.6)
Inoculated control (T2)	10.0 (18.4)	53.3 (46.9)	80.0 (63.9)	73.3 (60.0)	93.3 (77.7)
Brinkman pasteuriser (T3)	10.0 (18.4)	5.0 (9.2)	5.0 (9.2)	5.0 (9.2)	0.0 (0.0)
Priva UV 'Vialux' (T4)	10.0 (18.4)	10.0 (9.2)	5.0 (9.2)	5.0 (9.2)	5.0 (9.2)
Ozotech ozoniser (T5)	10.0 (18.4)	10.0 (18.4)	10.0 (18.4)	5.0 (9.2)	0.0 (0.0)
Memcor micro-filtration (T6)	5.0 (9.2)	5.0 (9.2)	10.0 (13.3)	5.0 (9.2)	0.0 (0.0)
Significance (of all treatments)	(**)	(***)	(***)	(**)	(***)
LSDs (5%) for comparing between:					
T1 and T2	(8.68)	(16.84)	(23.75)	(31.57)	(17.42)
T1 or T2 with T3-T6	(9.70)	(18.83)	(26.55)	(35.30)	(19.48)
T3, T4, T5 and T6	(10.63)	(20.63)	(29.09)	(38.67)	(21.34)

Angular transformation of data conducted for analysis. Transformed data is presented in parentheses.

NB. Confirmation based on the presence of smooth walled oospores consistent with those of *P. aphanidermatum* in the roots as observed using microscopy.

Table 10: Percentage recovery of *P. aphanidermatum* using a serological test (multi-well plate assay) on 'bait' cucumber seedlings at intervals following artificial introduction at a single focus in each plot.

Treatments	Date 12/3	8/4	30/4	21/5	18/6
Uninoculated control	0.0 (0.0)	10.0 (12.6)	70.0 (56.6)	100.0 (90.0)	100.0 (90.0)
Inoculated control	20.0 (26.1)	60.0 (53.5)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
Brinkman pasteuriser	10.0 (18.4)	10.0 (13.3)	15.0 (22.5)	35.0 (36.2)	10.0 (13.3)
Priva UV 'Vialux'	15.0 (22.5)	10.0 (18.4)	20.0 (25.8)	25.0 (28.8)	30.0 (33.2)
Ozotech ozoniser	20.0 (26.6)	10.0 (18.4)	35.0 (36.2)	35.0 (36.2)	10.0 (18.4)
Memcor micro-filtration	15.0 (22.5)	10.0 (13.3)	45.0 (42.0)	10.0 (13.3)	0.0 (0.0)
Significance (of all treatments)	(***)	(**)	(**)	(***)	(***)
LSDs (5%) for comparing between:					
T1 and T2	(8.82)	(22.15)	(27.16)	(16.38)	(12.50)
T1 or T2 with T3-T6	(9.96)	(24.76)	(30.36)	(18.31)	(19.98)
T3, T4, T5 and T6	(10.80)	(27.13)	(33.26)	(20.06)	(15.31)

Angular transformation of data conducted for analysis. Transformed data is presented in parentheses.

Table 11: Recovery (+/-) of *P. aphanidermatum* from nutrient solution in the collection tanks and pre and post-treatment tanks using microscopy on 'bait' seedlings following artificial introduction at a single focus in each plot.

	Date				
Treatments	12/3	8/4	30/4	21/5	18/6
<u>Collection Tanks</u>					
1. Uninoculated	-	+	+	+	+
2. Inoculated	-	+	+	+	+
3. Pasteuriser	-	+	+	-	+
4. UV	-	+	+	+	+
5. Ozoniser	-	+	+	+	+
6. Micro-filtration	-	+	-	+	+
<u>Pre-Treatment Tanks</u>					
7. Pasteuriser	-	+	-	-	+
8. UV	-	+	+	+	+
9. Ozoniser	-	-	-	-	+
10. Micro-Filtration	-	-	-	+	+
<u>Post-Treatment Tanks</u>					
11. Pasteuriser	-	-	-	-	-
12. UV	-	+	-	+	+
13. Ozoniser	-	+	-	+	-
14. Micro-filtration	-	-	-	-	+

\+ Positive confirmation of *P. aphanidermatum* based on visual observation of oospores conforming to *P. aphanidermatum* in the root tissues of 'bait' plants using microscopy.

Table 12: Recovery (+/-) of *P. aphanidermatum* from nutrient solution in the collection tanks and pre and post-treatment tanks using serological multi-well plate assays of 'bait' seedling roots following artificial introduction at a single focus in each plot.

Treatments	Date 12/3	8/4	30/4	21/5	18/6
Collection Tanks					
1. Uninoculated	-	-	+	+	+
2. Inoculated	-	+	+	+	+
3. Pasteuriser	-	+	+	+	+
4. UV	-	?	+	+	+
5. Ozoniser	-	+	+	-	+
6. Micro-filtration	-	+	+	+	+
Pre-Treatment Tanks					
7. Pasteuriser	-	-	+	+	+
8. UV	-	-	-	+	+
9. Ozoniser	-	-	+	+	-
10. Micro-Filtration	-	-	+	+	+
Post-Treatment Tanks					
11. Pasteuriser	-	-	-	+	-
12. UV	-	-	-	-	-
13. Ozoniser	-	-	-	+	-
14. Micro-filtration	-	-	-	+	-

\+ Positive confirmation based on duplicate wells above normal threshold (x + 3 x standard deviation).

? Positive confirmation based on a reading above the Agridiagnostics known positive but below the normal threshold.

Table 13: Assessments of wilting at intervals during the trial period following artificial inoculation with *P. aphanidermatum* at a single focus in each plot.

	Wilt Index (0-100)				
Treatments	13/4*#	15/4+#	30/4+#	7/5+#	7/6+
Uninoculated control	0.0 (0.00)	1.7 (1.21)	17.4 (3.67)	33.7 (5.37)	4.8
Inoculated control	3.5 (1.57)	13.3 (3.55)	18.5 (4.50)	34.8 (6.05)	0.7
Brinkman pasteuriser	0.3 (0.37)	1.4 (0.83)	2.8 (1.18)	2.8 (1.18)	0.0
Priva UV 'Vialux'	0.0 (0.00)	0.0 (0.00)	0.3 (0.37)	0.0 (0.00)	0.0
Ozotech ozoniser	0.0 (0.00)	0.0 (0.00)	2.2 (1.44)	0.6 (0.53)	0.8
Memcor micro-filtration	0.6 (0.53)	1.4 (1.12)	0.8 (0.65)	0.0 (0.00)	0.8
Significance (of all treatments)	(NS)	(**)	(**)	(***)	**
LSDs (5%) for comparing:					
T1 and T2	(-)	(1.62)	(1.76)	(2.28)	2.13
T1 or T2 with T3-T6	(-)	(1.80)	(1.97)	(2.55)	2.38
T3, T4, T5 and T6	(-)	(1.98)	(2.16)	(2.79)	2.61

\# Square root transformation of data prior to analysis. Transformed data in parentheses.

* Assessments conducted during dull, overcast weather.

\+ Assessments conducted during bright, sunny conditions.

Table 14: Assessments of stem disease on 17 June 1993.

	% Plants with Stem Disease		
Treatments	*B. cinerea*	*Sclerotinia sclerotiorum*	*Penicillium oxalicum*
Uninoculated control	9.5 (8.53)	1.1 (1.00)	3.3 (0.53)
Inoculated control	2.8 (3.69)	0.6 (0.67)	0.6 (0.03)
Brinkman pasteuriser	2.5 (2.50)	0.8 (0.84)	1.7 (0.00)
Priva UV 'Vialux'	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
Ozotech ozoniser	2.5 (2.50)	0.0 (0.00)	0.0 (0.00)
Memcor micro-filtration	0.0 (0.00)	0.8 (0.84)	0.0 (0.00)
Significance	(*)	(NS)	(NS)
LSD's (5%) for comparing:			
T1 and T2	(6.70)	(2.38)	(0.975)
T1 or T2 with T3-T6	(7.49)	(2.66)	(1.090)
T3, T4, T5 and T6	(8.20)	(2.92)	(1.194)

Analysis of variance performed on figures after adjustment for covariate. Mean figures are presented in parentheses.

Table 15: Assessment of stem disease and plant vigour at crop termination on 5 July 1993.

Treatments	Plant Vigour Index (0-100)	% Plants with: Botrytis	Sclerotinia
Uninoculated control	65.6 (66.70)	7.2 (6.60)	0.6 (0.60)
Inoculated control	74.8 (73.67)	2.2 (2.85)	0.6 (0.51)
Brinkman pasteuriser	92.2 (92.23)	1.7 (1.67)	0.8 (0.84)
Priva UV 'Vialux'	96.7 (96.67)	0.0 (0.00)	0.0 (0.00)
Ozotech ozoniser	92.2 (92.23)	3.3 (3.34)	0.0 (0.00)
Memcor micro-filtration	92.5 (93.50)	0.8 (0.84)	0.0 (0.00)
Significance (of all treatments)	(***)	(NS)	(NS)
LSDs (5%) for comparing:			
T1 and T2	(4.84)	(-)	(-)
T1 or T2 with T3-T6	(5.41)	(-)	(-)
T3, T4, T5, and T6	(5.92)	(-)	(-)

Analysis of variance performed on figures after adjustment for covariate. Mean figures are presented in parentheses.

Table 16: Assessments of root development and root discoloration at crop termination on 5 July 1993.

Treatments	Root Development Index (0-100)[+]	Root Discoloration Index (0-100)*
Uninoculated control	72.2 (72.2)	61.1 (60.6)
Inoculated control	65.5 (65.6)	95.6 (96.0)
Brinkman pasteuriser	91.7 (91.7)	32.5 (32.5)
Priva UV 'Vialux'	90.0 (90.0)	25.0 (25.0)
Ozotech ozoniser	92.5 (92.5)	23.3 (23.3)
Memcor micro-filtration	97.5 (97.5)	8.3 (8.3)
Significance (of all treatments)	(***)	(***)
LSDs (5%) for comparing:		
T1 and T2	(10.82)	(17.10)
T1 or T2 with T3-T6	(12.09)	(19.11)
T3, T4, T5 and T6	(13.25)	(20.94)

Analysis of variance performed on figures after adjustment for covariate. Mean figures are presented in parentheses.

[+] A high figure here indicates extensive root development.

* A high figure here indicates severe root discoloration.

Table 17: Yield data for the cucumber trial in House M20 (1993).

Treatments	Total Yield (kg/m^2)	Fruit (No/m^2)	% Class I of Total Yield
Uninoculated control	27.9	59.1	80.1
Inoculated control	29.4	61.2	78.8
Brinkman pasteuriser	34.8	72.7	80.6
Priva UV 'Vialux'	37.3	75.2	79.6
Ozotech ozoniser	37.4	74.8	79.9
Memcor micro-filtration	37.7	75.2	80.4
Significance (of all treatments)	***	***	NS
LSDs (5%) for comparing:			
T1 and T2	3.83	7.61	-
T1 or T2 with T3-T6	4.28	8.51	-
T3, T4, T5 and T6	4.69	9.32	-

Trial 3 (June - November 1993)

Seedling Bioassay

Following artificial inoculation on 10 August *F. oxysporum* f.sp. *lycopersici* was detected at 60% of the inoculation ports in the inoculated control on 31 August (Table 18). A lower recovery 20-40% was made in the inoculation ports of the various disinfection treatments at this stage and none was recovered from the uninoculated control ports.

By 12 October *F. oxysporum* f.sp. *lycopersici* was recovered sporadically from the vascular tissue of the 'bait' seedlings in all treatments, including the uninoculated control and in many cases at locations in the trial other than the inoculation point, providing some evidence of dispersal. However, there was no obvious pattern to the dissemination and this made interpretation of the results difficult when considered alone.

F. oxysporum f.sp. *lycopersici* was not recovered from any of the nutrient solution collection, pre or post-treatment tanks during the experiment using the 'bait' seedlings.

Multi-Well Serological Assay

No serological assay was available to test for *F. oxysporum* f.sp. *lycopersici* in this trial.

On-Site Serological Assay

No on-site assay kits were available to test for *F. oxysporum* f.sp. *lycopersici* in this trial.

Disease Aetiology

No symptoms of *F. oxysporum* f.sp. *lycopersici* became apparent in this wilt susceptible cultivar Ailsa Craig for some considerable time following inoculation. However, by early October occasional plants at the inoculation point in each treatment exhibited a pronounced yellowing of the lower leaves; often only one side of the plant or leaf being affected. Aseptic examination of the vascular tissues of affected plants revealed a pronounced 'foxy-red' vascular staining progressing throughout the plant (see Figure 11 in Appendix I). Tissue pieces, removed aseptically and plated out onto agar, confirmed *F. oxysporum* f.sp. *lycopersici* as the primary cause of these symptoms.

A disease assessment conducted on 14 October (Table 19) showed a small number of plants in most treatments to be exhibiting characteristic symptoms of Fusarium wilt. By early November, symptoms were more pronounced and a visual assessment on 5 November showed ca. 9% plants affected in the inoculated control, with some evidence of dispersal to plants remote from the inoculation point. In all disinfection plots symptoms were evident at the inoculation sites only at this stage. An assessment later in the month revealed a similar distribution of affected plants (Table 19). By crop termination of this late tomato trial in early December, ca. 28% plants were showing characteristic symptoms of infection (Table 20) in the inoculated control. Characteristic symptoms of *Fusarium* also occurred at low levels in the uninoculated control and this provided evidence that aerial spread may have occurred in the house. The symptoms of sporulating lesions high up the stem were consistent with this hypothesis (see Figure 12 in Appendix I). However, infection levels remained low and largely confined to the inoculation points following treatment of the recirculating hydroponic solution with the various disinfection equipment and this indicated that the primary, but not exclusive, means of dispersal was in the irrigation solution and that the disinfection equipment had been effective.

It was possible to conduct destructive assessments of the vascular tissue when the crop was terminated in December. Plants were scored for the presence/absence of vascular staining and samples returned to the laboratory for isolation onto agar. In the inoculated control 34.3% plants exhibited vascular staining and *F. oxysporum* f.sp. *lycopersici* was recovered consistently from the stained vascular tissue (Table 21). In comparison very few (0.3%) plants exhibited any staining in the uninoculated control and no *Fusarium* was recovered. In the plots receiving solution disinfected using the pasteuriser only 5% plants exhibited vascular staining. However, *Fusarium* was recovered from 10.7% of the stems in laboratory tests. Following UV disinfection 7% plants exhibited vascular staining and yet *Fusarium* was recovered from 14.7% plants on agar. These results suggest some plants in these treatments were latent carriers of *Fusarium* wilt. With ozone treatment 6.3% of plants exhibited staining and *Fusarium* was isolated from 6.9% of stems, and similarly following micro-filtration 4.7% of plants showed vascular staining and *Fusarium* was isolated from 5.9% of the stems.

These results were fairly well reflected in the overall appearance of the root tissues in the rockwool slabs in early December (Table 22). The root development index was reduced in the inoculated control and increased to a level above that of the uninoculated control with three of the disinfection treatments (ozone excluded). Root tissues in the uninoculated control exhibited some discoloration (mean index of 23.3) but this was significantly more pronounced in the inoculated control (41.3). Interestingly, the index of discoloration following disinfection was much lower than the uninoculated control with the exception of the ozone treatment.

Yield

Because this trial was of a short duration (July to December) there was relatively little opportunity to measure yield effectively and given the late establishment in the trial crop, yield was unlikely to provide an effective means of measuring disease and/or disinfection effects. The yield results are presented in Table 23 but no significant differences between treatments were recorded.

Table 18: Percentage recovery of *F. oxysporum* f.sp. *lycopersici* from 'bait' tomato seedlings, cv. Ailsa Craig, following artificial introduction at a single focus in each plot.

Treatment	Date		
	31 August[+]	12 October	24 November
Uninoculated control	0.0 (0.0)	3.3 (4.8)	0.0 (0.0)
Inoculated control	60.0 (54.0)	13.3 (16.7)	3.3 (4.8)
Brinkman pasteuriser	20.0 (18.0)	10.0 (14.5)	6.7 (7.1)
Priva UV 'Vialux'	40.0 (36.0)	23.3 (25.7)	0.0 (0.0)
Ozotech ozoniser	20.0 (18.0)	13.3 (19.3)	0.0 (0.0)
Memcor micro-filtration	20.0 (18.0)	23.3 (28.6)	3.3 (4.8)
Significance	(-)	(*)	(NS)
LSD (5%) for all comparisons	(-)	(8.08)	(-)

[+] 'Bait' tests were conducted at inoculation sites only (including equivalent sites in uninoculated control) and no statistical analysis was conducted.

NB. *Fusarium oxysporum* was not detected on any 'bait' seedling from the collection, pre- or post-treatment disinfection tanks during the study.

Table 19: Assessment of Fusarium wilt symptoms at intervals during the trial period.

Treatment	% Plants with Symptoms of Fusarium Wilt 14 October	5 November	17 November
Uninoculated control	0.0 (0.00)	0.0 (0.00)	0.3 (0.35)
Inoculated control	0.7 (0.66)	8.7 (8.71)	9.7 (9.65)
Brinkman pasteuriser	0.3 (0.33)	3.0 (3.04)	3.0 (2.98)
Priva UV 'Vialux'	1.7 (1.67)	3.3 (3.29)	3.0 (3.01)
Ozotech ozoniser	0.7 (0.66)	3.7 (3.71)	3.3 (3.32)
Memcor micro-filtration	0.7 (0.67)	2.7 (2.62)	3.0 (3.01)
Significance	NS	***	***
LSD (5%) for all comparisons	-	(1.654)	(1.822)

NB. Inoculation in this trial was conducted on 5% of the plants ie. one slab (or three plants) out of 20 slabs (60 plants) in each of five replicate plots. Figures above 5% therefore indicate that dissemination has occurred.

Analysis of variance performed on figures after adjustment for covariate. Mean figures are presented in parentheses.

Table 20: Assessment of Fusarium infection at crop termination on 3 December 1993.

Treatment	Mean No. of Plants/Plot with Fusarium Symptoms*	Index of Fusarium Infection (0-100)#
Uninoculated control	0.4 (-0.14)	0.4 (0.32)
Inoculated control	17.8 (6.74)	20.7 (4.48)
Brinkman pasteuriser	2.8 (2.34)	3.9 (1.94)
Priva UV 'Vialux'	3.8 (2.86)	4.7 (2.18)
Ozotech ozoniser	3.4 (1.54)	5.0 (2.15)
Memcor micro-filtration	2.2 (1.06)	3.6 (1.90)
Significance	(***)	(***)
LSD (5%) for all comparisons	(2.015)	(0.5844)

Square root transformation of data conducted prior to analysis. Transformed data is presented in parentheses.

* Analysis of variance performed on figures after adjustment for covariate. Mean figures are presented in parentheses.

Table 22: Assessment of root development and root discoloration in the slabs at crop termination on 3 December 1993.

Treatment	Index of Root Development (0-100)*	Index of Root Discoloration (0-100)+
Uninoculated control	81.3 (49.26)	23.3 (14.34)
Inoculated control	74.1 (44.34)	41.3 (24.46)
Brinkman pasteuriser	87.3 (51.94)	12.7 (7.26)
Priva UV 'Vialux'	82.7 (50.26)	12.7 (7.94)
Ozotech ozoniser	77.7 (45.74)	20.3 (11.86)
Memcor micro-filtration	85.7 (51.86)	13.0 (8.14)
Significance	(**)	(***)
LSD (5%) for all comparisons	(1.69)	(4.93)

Analysis of variance performed on figures after adjustment for covariate. Mean figures are presented in parentheses.

* A high figure indicates extensive root development.

\+ A high figure indicates severe root discoloration.

Table 23: Yield data for the tomato trial, cv. Ailsa Craig, inoculated with *F. oxysporum* f.sp. *lycopersici* (July- November 1993).

Treatment	Total Yield (kg/m^2)	% Class I of Total Yield	% Waste of Total Yield
Uninoculated control	8.56	47.2	33.2
Inoculated control	8.62	46.6	33.3
Brinkman pasteuriser	8.41	42.3	37.5
Priva UV 'Vialux'	8.78	42.2	35.2
Ozotech ozoniser	8.72	43.3	36.7
Memcor micro-filtration	8.73	45.3	33.0
Significance	NS	NS	NS
LSD (5%) for all comparisons	-	-	-

NB. Overall yield was low due to the relatively short duration of the trial.

DISCUSSION

Designing experiments to determine whether root pathogens are effectively prevented from disseminating in hydroponic solution, by disinfection of the solution, are fraught with difficulties, particularly when it is necessary to conduct the experiments on a large semi-commercial scale.

Previous work in this area has concentrated on determining the minimum or optimum dose or pore size for effective disinfection and much of the work has stopped short of 'commercial' evaluation. One of the primary aims of this project was to identify whether it was feasible to prevent dissemination of the primary root pathogens of both tomato and cucumber and thereby maintain commercial yields in treatments where these root pathogens had been introduced. In this respect the trials series has been very successful.

The tomato trial inoculated with the aggressive oomycete fungus *P. cryptogea* provided an excellent demonstration of how readily the fungus is dispersed via the irrigation water. It has also highlighted the importance of designing collection channels effectively to prevent rooting out into the collection channel beneath and thereby negating the full benefits of disinfection.

Pathogen detection methods are limited and the use of a 'bait' system here using host seedlings alongside ELISA technology (Multi-Well assay) provided an interesting comparison. Both techniques were largely effective in monitoring dispersal, the former being rather labour-intensive requiring effective, trained technicians to recognise the characteristic sporangia of the pathogen on the root issues. The latter, however, whilst providing an opportunity for processing large numbers of samples is rather expensive and, on occasions, produced apparently aberrant results.

The bioassay test appeared to give few false positive reactions and confidence was quickly gained in interpreting the results from these tests effectively. The results from these tests mirrored closely the development and expression of characteristic disease symptoms in the crop.

The Multi-Well serological assay, however, relied on a colour change above a pre-determined threshold in duplicate wells for confirmation of pathogen presence. In situations where the colour change was close to, but below, the threshold and where only one of the two duplicate wells gave a positive reaction it became difficult to interpret the result. This was highlighted very effectively in the nutrient solution tanks 'baited' with tomato root tissue, where on three separate occasions one of the duplicate wells only, gave a positive reaction, including in the post-treatment tanks. These results were almost certainly false positives as the crop exhibited no outward signs of *Phytophthora* infection following solution disinfection.

The Alert on-site kits were largely ineffective in detecting the presence of *P. cryptogea* in this trial and their use cannot therefore be recommended as a means of checking for the presence of early infection by *P. cryptogea in situ* in hydroponic tomato crops. None of the detection systems evaluated provided a measure of determining or quantifying the propagule type (eg zoospores or oospores) in solution and yet this may be very important with regard to disease progression in the crop.

Ultimately, whilst the monitoring of pathogen dissemination was open to some interpretation it was possible to rely on the crop itself to provide a reliable indicator of pathogen dispersal.

From disease assessments conducted it became clear that *P. cryptogea* spread rapidly to infect almost all plants in inoculated plants control. The total lack of symptoms in the uninoculated highlighted the effectiveness of trial design and hygiene standards and the pathogens inability to be disseminated by means other than via the irrigation solution. Indeed, were it not for the fact that occasional plants rooted out of the growing media (slabs) into the collection solution, the pathogen would have been confined to the point of introduction following disinfection with both heat and UV. Therefore both disinfection systems can be recommended for use in tomatoes for the control of this root pathogen. It must be considered, however, that with the exception of *F. oxysporum* used

in Trial 3 no evaluations have been conducted to determine the performance of the test equipment against other root pathogens in this crop, eg. *Pyrenochaeta lycopersici, Humicola fuscoatra, Thielaviopsis basicola, Colletotrichum coccodes, Verticillium* spp., bacterial and virus diseases.

Perhaps the most interesting observation in this trial was the relative small yield reduction following widespread dissemination of *P. cryptogea* in the inoculated control plots. It is not uncommon to find *P. cryptogea* in NFT tomato crops (McPherson and Scrace, 1994) where there is ample opportunity for dispersal and subsequent widespread root infection. Yet, the combined effects of this pathogen in conjunction with other root infecting fungi (eg *T. basicola, C. coccodes)*, rarely cause yield losses in excess of 10% in this crop. This observation has led to the suggestion that there may be a natural 'suppressive' phenomenon occurring in such hydroponic crops using recirculation technology preventing the aggressive development of root pathogens and ultimately suppressing any potentially large loss of yield. Yield losses in trials on tomato where a run-to-waste hydroponic regime has been adopted has been as high as 60% where *P. cryptogea* has been introduced (McPherson, pers comm).

The cucumber trial inoculated with the aggressive oomycete fungus *P. aphanidermatum* also provided an excellent demonstration of how readily the fungus is dispersed via the irrigation water. In this trial Brinkman 'aquatrays' were used to ensure rooting out was prevented between each group of three plants in each plot. Yet, the pathogen was observed to be disseminated by means other than via the irrigation water and the fungus was quickly found in uninoculated control plots. Similar observations to this have been recorded previously with this pathogen on cucumber (McPherson, pers comm). The most likely explanation for this air-borne dissemination is via sciarid or shore flies in the growing house. Measures for sciarid fly prevention need to be sought with some urgency if this pathogen is to be contained both in experimental programmes and in commercial crops. It is interesting and perhaps surprising that *P. cryptogea*, a similar oomycete fungus, does not appear to be transmitted in the same manner.

Following widespread dispersal of *P. aphanidermatum* in the inoculated control plots of this cucumber trial, wilt symptoms ensued and by early May many plants were severely affected. However, by early June the crop appeared to recover and wilt symptoms did not recur even under bright light conditions. It is considered that this apparent amelioration of wilt symptoms, in the absence of disinfection, may be similar to that observed in the earlier tomato trial and certainly warrants further investigation.

Monitoring of pathogen dissemination using 'bait' seedlings was effective though the presence of other structures in the root tissues eg. *Olpidium* spp. meant that considerable care had to be taken before recording a positive result for *P. aphanidermatum* oospores. A progressive increase in recovery was achieved in both Treatments 1 and 2 and this occurred in advance of disease aetiology. Similarly, a low recovery was achieved following disinfection and these plots remained healthy throughout. This provided a clear unambiguous result to demonstrate the effectiveness of disinfection using heat, UV, ozone, micro-filtration to effectively prevent dissemination of *P. aphanidermatum* in cucumber. The only ambiguous results were gained from seedling bait tests in the post-treatment disinfection tanks where occasional positive confirmations were made. Mis-identification of *P. aphanidermatum* oospores may be the reason for these false positive results and this demonstrates the need for skilled technicians in this type of study. A simpler, reliable technique for pathogen detection should be given high priority in future R & D programmes.

The serological multi-plate assay proved less reliable. Whilst positive confirmation of *P. aphanidermatum* in the uninoculated (but infected) and inoculated controls was higher than with the 'bait' assay, frequent positive readings in the four disinfection treatments suggested that dissemination had occurred. Yet ultimately, the crop in Treatments 3-6 appeared healthy and continued to remain disease-free for the duration of the experiment.

When this crop was terminated a most striking result was recorded. Plants were excised and the rockwool slab inverted in order to assess both the quantity and quality of the root tissues at the base. Root development in Treatments 1 and 2 was poorer than in any of the disinfection treatments and the roots present at the base of the rockwool slabs had a high level of discoloration. However, whilst there remained a moderate amount of discoloration following disinfection with heat, UV and ozone, the roots in the micro-filtration treatment (with the exception of the inoculated slabs) remained remarkably healthy and white, and this provided a stark contrast between the treatments (Figure 10).

It has already been proposed, following observations in the earlier tomato trial, that there may be biological factor operating causing disease suppression. This may also be the case in cucumbers. The results presented in this report provide further evidence in this respect but also support the hypothesis that the effect may be negated following some forms of disinfection.

If a biological suppression of root disease does occur naturally in hydroponic solution then:

(a) the effect will be less pronounced in run-to-waste systems where excess solution (and its contents) is discarded.

(b) 'active' disinfection systems, eg. heat, UV, ozone, will potentially destroy the biological factors and therefore negate any natural suppression.

(c) 'passive' disinfection, eg. filtration, may potentially remove a large proportion of the pathogen propagules but at the same time, maintain a 'biological equilibrium' in solution.

In the third trial on tomato *F. oxysporum* f.sp. *lycopersici,* cause of vascular wilt, was used to substitute for *F. oxysporum* f.sp. *radicis-lycopersici* cause of crown and root rot of tomato, as growers in the North of England were reluctant to encourage its further distribution in the UK. The wilt pathogen was used on the understanding that the results obtained using the disinfection could probably be applied equally well to the crown and root rot pathogen.

Unfortunately, however, whilst symptoms caused by *F. oxysporum* f.sp. *lycopersici* were slow to develop when they did occur they produced aggressive sporulating lesions along the stem. It therefore became difficult to interpret results of this trial accurately because of the potential for air-borne and worker transmitted dissemination in the growing house.

Fortunately, only localised pockets of infection appeared in the uninoculated control and therefore it was reasonable to assume that the higher incidence in the inoculated control was due to transmission via the irrigation solution rather than via an air-borne source. Using this interpretation it was evident that the disease spread to infect over 30% plants in the inoculated control by termination in early December. In contrast the pathogen was largely restricted by the various disinfection equipment and whilst some dispersal appears to have occurred, particularly with the UV and heat disinfection, some could have been accounted for by localised air/worker-borne spread. It would be necessary to repeat this trial using *F. oxysporum* f.sp. *radicis-lycopersici* where the opportunity for air-borne spread is much reduced, for complete confidence with any of the disinfection results.

There is no doubt that the results from these trials have demonstrated, particularly with the oomycete pathogens, that both tomato and cucumber crops can be grown effectively in a circulating hydroponic environment, providing a disinfection installation is utilised. The ultimate choice between the disinfection techniques tested does not appear to be too critical and the initial price and

subsequent running costs may be governing factors. It has however not been possible to provide prices and running costs in this report because the figures would not reflect those of a commercial unit where significantly larger water volumes are involved and they would not be operated on a batch treatment basis. A guide to comparative capital and running costs of heat, ozone and UV disinfection systems is presented by O'Neill and Berry in HDC report CP4.

It should also be noted here that, whilst relatively little difference has been detected between the units tested in this series of experiments, it does not guarantee their effectiveness against the diverse array of other root pathogens of these crops. These would need to be tested in subsequent studies.

Finally, in view of the fact that an opportunity has been identified for the development of a biologically sustainable technique for root disease suppression, the author is of the view that, in the UK at least, where pressure to recirculate is less advanced than in Holland, it is premature in most cases to consider immediate investment in disinfection technology. A new MAFF LINK project has been agreed and is now underway to answer some of the questions posed regarding other root pathogens of protected salad crops and to investigate the observed biological suppression. Once information from this project becomes available the advantages and disadvantages of 'active' and 'passive' disinfection will hopefully become much clearer.

CONCLUSIONS

- In the absence of disinfection the root pathogens *P. cryptogea* and *F. oxysporum* f.sp. *lycopersici* were disseminated via the hydroponic solution in two tomato crops during 1992 and 1993 respectively.

- In the absence of disinfection the root pathogen *P. aphanidermatum* was disseminated via the hydroponic solution in a cucumber crop during 1993.

- *P. aphanidermatum* was observed to spread to the uninoculated control by means other than in the hydroponic solution. Sciarid or shore flies were regarded as the most likely culprits for this aerial dispersal. Specific control measures for these flies in the hydroponic environment need to be determined.

- *F. oxysporum* f.sp. *lycopersici* produced severe symptoms on the cv. Ailsa Craig and sporulation was extensive along the stem of some infected plants. Results from this trial therefore need to be interpreted with caution.

- In 1992, both the heat and UV systems of disinfection evaluated were very effective in preventing dispersal of *P. cryptogea* in the irrigation solution.

- In 1993, heat, UV, ozone and micro-filtration were all effective in preventing dissemination of *P. aphanidermatum* via the irrigation solution in cucumber.

- Heat, UV, ozone and micro-filtration appeared to largely prevent the dispersal of *F. oxysporum* f.sp. *lycopersici* though there was some doubt over the results for the pasteuriser and the UV equipment. This was compounded by the potential for air-borne dispersal in this trial.

- The yield loss in the tomato trial in 1992, as a result of *P. cryptogea* infection, was lower than expected. This has led to the hypothesis that a biological suppression occurs in hydroponic solution (in the absence of disinfection).

- Whilst the yield loss in the cucumber trial following infection by *P. aphanidermatum* was higher than that observed in the earlier tomato trial, the crop was observed to recover and this again suggested a natural or 'biological' amelioration of root disease.

- Results of disinfection with micro-filtration in the cucumber trial were quite outstanding, the technique retaining the roots in a near perfect condition. This provides further support to the view that a biological suppression occurs and adds weight to the concept of 'passive' disinfection to retain the biological effects rather than 'active' systems which will potentially negate any biological effect.

- None of the disinfection equipment had a particularly adverse effect on the nutrient status of the solution. The UV system caused a slight reduction in iron due to deposition on the quartz tube. As the reduction in iron only occurred in the run-off solution the imbalance could be quickly corrected with the fresh water/nutrients.

- A new MAFF LINK project has commenced to further investigate the potential of disinfection systems for root disease control and to attempt to investigate some of the more fundamental hypotheses posed during this project.

ACKNOWLEDGEMENTS

This project was funded by the Horticultural Development Council and their assistance in this respect is gratefully acknowledged. Particular thanks go to Dr N Dungey, Van Heyningen Bros Ltd, who acted as Project Co-ordinator for the study, and also Dr T M O'Neill, ADAS Cambridge for his useful discussion and critical comments on the manuscript.

The disinfection equipment was kindly loaned free of charge or offered on low cost rental terms by the manufacturers and the assistance and technical advice from Priva UK, Brinkman UK, Alfa-Laval Agri, Ozotech UK, and Memcor Ltd was kindly appreciated.

Some of the initial work in this project was conducted whilst the author was employed by ADAS Leeds and the assistance of both ADAS and HRI personnel is acknowledged. The professionalism and technical enthusiasm by which they undertook the work is to be commended. Analysis of the data was performed in conjunction with colleagues from the Biometrics department at Wellesbourne. The assistance and patience of Mr R Edmondson and Miss A Jackson was appreciated.

The culture of *F. oxysporum* f.sp. *lycopersici* and the seed cv. Ailsa Craig were kindly supplied by Dr J T Fletcher (formerly ADAS Wye) and Mr B Sparkes, English Village Nurseries (formerly L O Jeffs) respectively and their technical assistance and support throughout this project was much appreciated.

REFERENCES

Anon. (1990). Essentials. Multi Year Crop Protection Plan. Information leaflet, The Hague, August 1990

Anon. (1993). Ozone as a disinfectant - A review. Ozotech Ltd, Burgess Hill, London.

Bates, M. L. & Stanghellini, M. E. (1984). Root rot of hydroponically grown spinach caused by *Pythium aphanidermatum* and *P. dissotocum*. Plant Disease, 68, 989-991.

Daughtrey, M., & T. Weller. (1992). Managing diseases in recirculating systems. Grower Talks, February, 59-71.

George, R. K.; Biernbaum, J.; Heins, R. & W. Carlson. (1989). Water management and plant pathogens: Do diseases really run rampant in recirculating systems? Grower Talks, September, pp 65-67.

Goldberg, N. P., & Stanghellini, M. E. (1990). Ingestation - egestion and aerial transmission of *Pythium aphanidermatum* by shore flies (Ephydrinae: Scatella stagnalis). Phytopath 80, 1244-1246.

Goldberg, N. P.; Stanghellini, M. E. & S. L. Rasmussen. (1992). Filtration as a method for controlling Pythium root rot of hydroponically grown cucumbers. Plant Disease 76, 777-779.

Jarvis, W. R.; J. L. Shipp, & R. B Gardiner. (1993). Transmission of *Pythium aphanidermatum* to greenhouse cucumber by the fungus gnat *Bradysia impatiens* (Diptera: Sciaridae). Ann. Appl. Biol. 122, 23-29.

Jarvis, W. R. (1990). Understanding fungus gnats. Grower 22 March, pp 37-38.

Jenkins, S. F. Jr. & C. W. Averre. (1983). Root disease of vegetables in hydroponic culture systems in North Carolina greenhouses. Plant Disease, 67, pp 968-970.

McPherson, G. M. (1992). Disease dictated decision. In: The Grower, August 6 1992, pp 22-24.

McPherson, G. M. (1993). Recirculation - A pathologists perspective. Abstract for National Cucumber Conference, 6 October 1993.

McPherson, G. M. (1993). Is disinfection the answer to disease control. Abstract for National Tomato Conference, 22 September 1993.

McPherson, G. M. & M. R. Hardgrave (1993). Cleaning out root disease. In: The Grower, August 19 1993, pp 17-18.

McPherson, G. M. (1994). The perceived threat from root disease prevents widespread adoption of recirculation systems. Abstract for National Tomato Conference, 5 October 1994.

McPherson, G. M. & J. M. Scrace (1994). A survey of root pathogens in NFT and substrate-grown (run-to-waste) tomato crops. Final report for Horticultural Development Council, PC60, 38 pp.

McPherson, G. M., & M. R. Harriman (1995). Is recirculation too radical? In: HDC Project News, No. 33, June 1995, pp 6-7.

McPherson, G. M., M. R. Harriman, and D. Pattison. (1995). The potential for spread of root disease in recirculating hydroponic systems and their control with disinfection. Med Fac. Landbouww. Univ. Gent. 60/2b pp 371-379.

O'Neill, T. M., & A. M. Berry. . (1992). Disinfection in commercial horticulture: A review of chemical disinfectants, soil treatment with Formalin and water treatment for controlling plant pathogens. HDC report CP4.

Paludan, N. (1985). Spread of viruses by recirculated nutrient solutions in soilless cultures. Tidssker, Planteavl, 89, 467-474.

Rattink, H. (1989). Annual Report Research Institute for Plant Protection, 1988, 21.

Rattink, H. (1991). Epidemiology of Fusarium Crown and Root rot in artificial substrate systems. Med. Fac Landbouww, Rijksuniv, Gent, 56, 423-430.

Rattink, H. (1990). Epidemiology of Fusarium wilt in cyclamen in an ebb & flow system. Neth. J. Pl. Path. 96, 171-177.

Rattink, H. (1983). Spread and control of Fusarium wilt in carnations on artificial substrates. Acta Hort. 141, 103-109.

Runia, W, Th. (1993). A review of possibilities for disinfection of recirculation water from soilless cultures. In Proc. Leuven.

Runia, W, Th.; Van Os, E. A. & G. J. Bollen. (1988a). Disinfection of drainwater from soilless cultures by heat treatment. Netherlands J. of Agric. Science, 36, 231-238.

Runia, W. Th. (1988). Elimination of plant pathogens in drainwater from soilless cultures. In: ISOSC (Proc), 7th Int. Congr. on Soilless Culture, pp 429-443.

Sanogo, S. & G. W. Moorman. (1993). Transmission and control of *Pythium aphanidermatum* in an ebb and flow subirrigation system. Plant Disease, 77, 287-290.

Stanghellini, M. E.; Stowell, L. J. & Bates, M. L. (1984). Control of root rot of spinach caused by *Pythium aphanidermatum* in a recirculating hydroponic system by ultraviolet irradiation. Plant. Dis. 68, 1075-1076.

Stanghellini, M. E., & S. L. Rasmussen. (1994). Hydroponics. A solution for zoosporic pathogens. Plant Disease 78, 1129-1138.

Staunton, W. P. & Cormican, T. P. (1978). The behaviour of tomato pathogens in a hydroponic system. Acta. Hort. 82, 133-135.

Thomlinson, J. A. & Faithfull, E. M. (1979). Effects of fungicides and surfactants on the zoospores of *Olpidium brassicae*. Annals of Applied Biology, 93, 13-19.

Wohanka, W. (1990). Gesschlossone Kulturverfahren im Zierpflanzenbau aus Sicht des pflanzenschutzes. Taspo Praxis 18, Geschlossene Kulturverfahren Zierpflanzenbau, 57-61.

Wohanka, W. (1993). Slow sand filtration and UV radiation; low cost techniques for disinfection of recirculating nutrient solutions or surface water. ISOSC Proc. of 8th Int. Congress. on Soilless Culture, 1992, 497-511.

Vanachter, A; Lieve Thys, E; Van Wambeke, E. & Van Assche, C. (1988). Possible use of ozone for disinfection of plant nutrient solutions. Acta Horticulturae 221: 295-301.