Effective management of root diseases in hydroponic lettuce

Len Tesoriero
NSW Department of Primary Industries (NSW DPI)

Project Number: VG04012

VG04012

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the vegetables industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the vegetable industry.

ISBN 0 7341 1746 9

Published and distributed by:

Horticulture Australia Ltd

Level 7
179 Elizabeth Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399

E-Mail: horticulture@horticulture.com.au

FINAL REPORT

Effective management of root diseases in hydroponic lettuce

HAL Project VG04012

Len Tesoriero *et al.*
Elizabeth Macarthur Agricultural Institute, Menangle

April 2008

HAL VG04012

Project Leader:

Len Tesoriero, Industry Leader: Greenhouse & Ornamental Crops
Elizabeth Macarthur Agricultural Institute, Menangle
PMB 8 Camden 2570
len.tesoriero@dpi.nsw.gov.au

Key Personnel:

Dr Leanne Forsyth, Plant Pathologist, EMAI, Menangle
Mr Jeremy Badgery-Parker, Extension Officer, HRI, Gosford
Mr Roger Carrus, Technical Officer, EMAI, Menangle
Mrs Fiona Lidbetter, Technical Officer, HRI, Gosford
Mr Josh Jarvis, Technical Officer, HRI, Gosford
Ms Aida Ghalayini, Technical Officer, EMAI, Menangle
Dr Mary Ann Terras, Technical Officer, EMAI, Menangle
Ms Brenda Gorrie, Technical Officer, EMAI, Menangle
Ms Teghan Crowe, Technical Assistant, EMAI, Menangle

The purpose of this project was to develop effective and economic management strategies for root diseases of hydroponic lettuce.

Acknowledgement

The research team wish to thank Rijk Zwaan Seeds Australia and Leppington Speedy Seedlings for providing seeds or transplants. Zadco for Quality Gro Pty Ltd funded some efficacy trials with Fulzyme Plus™ and other microbial biocontrols. Other companies (listed in the body of the report) provided their respective disinfectants, disinfecting equipment or other microbial biocontrols. Collaboration is acknowledged with Dr Khalaf Alhussaen who completed his doctorate study on Pythium and Phytophthora Root Rots of hydroponic lettuce with The University of Technology, Sydney in 2006. Technical support is acknowledged from the NSW DPI Plant Health Diagnostic Service, Menangle. Finally, we would like to thank hydroponic lettuce growers in NSW, Queensland, South Australia and Victoria who provided access to their farms and provided plant samples for disease surveys.

Table of Contents

List of tables and figures

Media Summary

Hydroponics is a quick, clean and efficient production system for leafy lettuce. The industry has a farm gate value of over $50 million annually. Growers are spread across Australia providing a fresh supply of lettuce to central and local markets.

Root rot diseases occur sporadically and hamper production efficiency. They cause major losses in hot weather and are an impediment to the expansion of the industry. This report details research into characterising the causal pathogens, confirming the major environmental factors that contribute to root rot disease expression and identifies effective ways to manage them.

One highlight has been to demonstrate consistent disease suppression by a strain of the bacterium, *Bacillus subtilis* formulated as a microbial biocontrol product. This product is being developed for registration as a bio-pesticide.

Disinfection strategies were evaluated but have strong limitations. Chemical disinfectants are toxic to roots at concentrations required to kill pathogens so their use should be restricted to non-crop use. Some were toxic to plant roots at even lower concentrations. They have an important role for farm and source water sanitation. UV-light and sonication were effective disinfection strategies for recirculated nutrients but they are expensive and require infrastructure changes to farms.

Since higher nutrient temperatures were generally associated with greater disease, finding economical and practical ways to maintain them at lower temperatures remains a challenge. Evaporative coolers and passive heat exchanger coils placed in nearby dams are being used commercially but they have limited effect under extended hot weather conditions. Growers also use shading, plastic screens or overhead watering to reduce heat stress. All integrated crop management strategies require growers to tailor those that are practical and suitable to individual production systems and geographic locations.

Some good disease management strategies are universal though. Poor farm and crop hygiene were strongly correlated with increased disease. It is most important to start production with healthy and uninfected seedlings and diseased plants should be removed and disposed of, both timely and hygienically.

This project has increased our understanding of these root rot diseases and has identified practical and effective management strategies. Further work could expand the suite of potential microbial biocontrols and refine their use-patterns to optimise disease control.

Technical Summary

Root rot diseases cause seasonal, sporadic and sometimes entire crop losses in Australian hydroponic lettuce crops. This project characterised the pathogens responsible, and determined their relative importance and distribution. Two Oomycetes were mostly associated with diseased roots. *Phytophthora cryptogea* was the most aggressive pathogen confirmed by pathogenicity assays. *Pythium coloratum* and closely related species were very common in roots, but only a few isolates were demonstrated to cause disease symptoms. Both *Pythium* and *Phytophthora* were commonly isolated from symptomless root samples throughout the year emphasising the relationship between disease expression and certain plant stresses. Seedlings (particularly those grown on the same site as the hydroponic lettuce production) were sometimes infected with pathogens suggesting one potentially significant means of entry into recirculating nutrient systems.

High nutrient temperature correlated positively with disease expression. It was the most important factor associated with extensive or entire crop losses. Reducing nutrient temperatures during periods of hot weather remains problematic and costly in electrical energy. A number of strategies identified in this project are being used commercially and with success. Some growers run their nutrient solutions through fan-assisted evaporative coolers. Others run the return nutrient lines via heat-exchange coils in nearby dams. Shade-cloth, plastic covers or overhead irrigation were identified as further management options to reduce nutrient temperatures and plant stress.

There was a large variation in lettuce cultivar susceptibility to root rot diseases. The cultivars Brown mignonette, Murai and Red Ferrari were the most susceptible tested. Other cultivars were only affected under conditions of plant stress.

Moisture stress (induced by stopping the flow of nutrients) and infection with Tomato spotted wilt virus were other factors that correlated with greater root rot disease expression. This emphasises the need for maintenance of infrastructure, hygienic cultural practices and effective pest management.

Poor hygiene and crop management practices were common on certain farms where disease and associated crop losses were greater. Examples of such practices were: discarding diseased plants on the ground under the channels; poor seedling production hygiene allowing early infections; and growing plants of different stages of maturity in the same system thereby allowing younger plants to become infected from the older ones.

Use of larger seedling plug sizes were shown to result in larger plants in the presence of plant pathogens, however further evaluation is required under conditions of high disease pressure.

A number of methods were assessed for water disinfection: various chemical disinfectants; sonication; and UV-light. Results with disinfectants indicate that concentrations and exposure times that are efficacious to plant pathogens are

phytotoxic to plant roots. Therefore they are best restricted to disinfecting tanks and channels between crops. They may also be used to disinfect source water in a tank (if required) but allowed standing time to dissipate before exposing to plant roots. Both UV-light disinfection units were highly efficacious but their cost is likely to be prohibitive except on large farms. Most farms employ several separate nutrient tanks that would multiply costs. Centralising a nutrient tank raises risks of losses through mechanical breakdowns, disease spread and affords less flexibility for periodic maintenance and disinfection. Other water disinfection options such as treatment with peroxide and ozone were not studied in this project but have similar limitations to those chemical disinfectants that were tested.

A more promising approach to controlling Pythium and Phytophthora root rots was the efficacy of certain microbial biocontrols in a series of replicated trials. One particular commercial product containing a strain of the bacterium *Bacillus subtilis* consistently suppressed disease expression to a level equivalent to the uninfected control treatments. It appeared to reduce the colonisation of roots by the pathogen. In some trials it stimulated plant growth even in the absence of the pathogen. Of the other potential biocontrols assessed, *Pseudomonas putida* and *Streptomyces lycius* were shown to give intermediate control of root rots. These and other potential biocontrols should be tested further and their compatibility assessed as mixed formulations.

Hydroponic NFT production of lettuce is an excellent model system to study root rots and their suppression by beneficial microbes. Chemical control options for root diseases leave undesirable residues and are therefore not permitted. Microbial biocontrols offer a sound alternative, providing they are used with other management strategies identified in this project.

Introduction

The Australian hydroponic lettuce industry has been estimated to comprise some 1000 growers on 242ha and with a gross farm gate value of $44.9 million (Anon., 2001). There are no recent and reliable production data to assess the current size of the industry. Almost all production occurs in recirculated nutrient systems (*Nutrient Film Technique* [NFT]) comprised of white PVC channels linked to a sump tank by plastic irrigation lines. Formulated nutrient is pumped through supply lines to suspended plant roots at approximately 1ml/sec. Channels are sloped to allow nutrient to flow by gravity and return to the sump tank. Hydroponic production enables productivity gains per unit area in the order of 15 times that of field production. The use of recirculated nutrient systems and the general water efficiency obtainable in hydroponics compared with soil production makes this industry highly productive on a water resource basis.

Root diseases can have a major impact on crop health and consequently production when they establish in a hydroponic system. Losses are often up to 20-30% and complete crop losses often occur during the summer period (Tesoriero *et al*. 1991). Internationally, there are several reports of root diseases in hydroponic lettuce production (reviewed by Stanghellini & Rasmussen, 1994). In Australian hydroponic lettuce, the water moulds, *Pythium* and *Phytophthora* and the fungus, *Thielaviopsis* have been reported to cause root diseases (Tesoriero *et al*., 1991, Hutton & Forsberg, 1991, & O'Brien & Davis, 1994). For many of these pathogens, especially *Pythium* and *Phytophthora*, there is also a relationship between nutrient solution temperatures and disease severity (Tesoriero & Cresswell, 1995). A wilt disease of lettuce crops has been described overseas (Japan, USA, Italy, Iran and Taiwan) caused by sub-species of the fungus *Fusarium oxysporum* (Matuo & Motohashi, 1967). No studies have determined if this disease is present in Australia. Given the propensity for several pathogens to occur together in diseased plants, this disease agent may have been overlooked in Australian lettuce production. The potential for spread of *Fusarium* with seeds increases the risks that this disease will enter Australia.

A range of growth stimulants and oxidising products are commonly used as a last resort to save crops and generate a marketable crop. The efficacy and value of these products has not been validated. Some growers have tried to use disinfectants in nutrient solutions with growing crops. Guidelines on preventative strategies to effectively manage these diseases are not unavailable.

Microbial biocontrol products consist of formulated fungal or bacterial inocula and have potential in an integrated disease management program. They are becoming available to the industry, but have not been assessed objectively for hydroponic lettuce production.

Many hydroponic lettuce producers rarely disinfect their recirculated nutrient solutions due to lost production through downtime. During high disease pressure growers rely on regular dumping of solutions, which could have

adverse environmental effects if nutrients enter waterways. Use of unregistered chemicals in nutrient solutions may also increase food safety and environmental risks. Recent developments in ultrasonic and ultraviolet disinfection in food, medical and wastewater industries may have application to recirculated hydroponic systems. They need to be adapted and validated for this industry.

Aims:

- Determine the current status of root diseases in Australian hydroponic lettuce crops from crop surveys.
- Characterise the pathogens, and determine their relative importance and distribution.
- Study the relationship between disease expression and nutrient temperatures.
- Evaluate economic strategies to reduce nutrient temperatures.
- Assess commercial lettuce cultivars for their relative susceptibility to the identified pathogens.
- Assess larger seedling plug sizes for their ability to tolerate pathogens.
- Test the efficacy and value of a range of disinfectants, UV light, ultrasonics, and filtration systems.
- Evaluate potential microbial biocontrols (particularly bacteria of the genera *Pseudomonas, Streptomyces* and *Bacillus*) and 'biostimulant' chemical formulations for their ability to suppress diseases.

Materials & Methods

1. Farm surveys & laboratory diagnosis

1.1 Farm surveys

Production surveys were used to determine currently important pathogens in four Australian states. Fourteen commercial hydroponic lettuce farms from the Sydney Basin and Central Coast of NSW were surveyed regularly over an 18-month period between September 2005 and March 2007. Further surveys and samples were collected from enterprises in Queensland, Victoria and South Australia.

1.2 Laboratory diagnosis

Root samples were washed and plated to semi-selective agar media (potato carrot agar [PCA] amended with pimaricin [@5ppm], rifampicin [@10ppm] and +/-hymexazole [@50ppm]). Plates were incubated at 25^{o}C and examined over a 7-day period for mycelial growth. Light microscopy (x100-200) was used to locate growth on agar plates that was then sub-cultured to PCA. Cultural and morphological features on agar media were initially used to identify taxa to genus level. Further morphological and molecular characterisation of selected isolates was used to confirm and distinguish taxa with similar morphologies. The key of Plaats-Niterink (1981) was used to identify species of *Pythium*. Burgess *et al*. (1994) was used to characterise *Fusarium* isolates. Sequences of internal transcribed spacer (ITS) regions from ribosomal RNA genes were compared with GenBank databases and similarity analysis was used to place isolates into discrete taxa. Details of these methods are noted below:

1.2.1 DNA extraction from isolates

Fungal cultures were grown in 100 mL of autoclaved half strength potato dextrose broth (Difco) in sterile 250 mL Erylenmeyer flasks for one weeks at 23^{o}C, being agitated twice daily. Mycelia were collected on two layers of miracloth (Calibiochem, La Jolla, USA), and stored at –20^{o}C.

DNA was extracted from the mycelia as per Bentley and Bassam (1996). DNA concentrations and quality were determined spectrophormetrically using a Nanodrop (Beckman-Coulter, Fullerton, USA) and directly on agarose gels.

1.2.2 Genetic analysis of isolates

To confirm the identity of the fungal isolates, PCR was performed using the fungal ITS primers ITS1 and ITS4, as described by White *et al*. (1990) were used. To confirm the identity of the oomycota isolates PCR was performed using the oomycete ITS primers TW81 and AB28, as described by Howlett *et al*. (1992). The PCR reaction was performed in a PTC-100 PCR machine (MJ Research, Watertown, USA). PCR products were examined electrophoretically on agarose gels stained with ethidium bromide. Excess primer and salts were removed from the end PCR product using a clean up kit (Ultraclean PCR, Mo Bio, Solana Beach, CA, USA), as per the manufacturer's instructions. Sequencing reactions, in the forward and reverse directions were

performed on the cleaned PCR product using BigDye3 (Applied Biosystems, Foster City, USA) as per the manufacturer's instructions.

The forward and reverse sequencing reactions of the PCR products were aligned and corrected manually using the sequence editor and aligner available in BioEdit version 7.0.5.3 (Hall, 1999). The resulting ITS gene sequences for the different isolates were aligned using BioEdit and compared to fungal isolates from the public database, using BLAST (Altschul *et al*., 1990).

2. Experiments to determine pathogenicity & product efficacy

In total, 25 experiments determined the relativity pathogenicity and efficacy of microbial biocontrols, growth stimulants and disinfectants for isolates collected during the farm surveys. Two experimental units were used; one located on NSW DPI site at Gosford on the NSW Central Coast (40 independent units [100L tanks] @ 40 plants per channel), and the other at EMAI, Menangle in the Sydney Basin (50 independent units [20L tanks] @ 12 plants per channel). Trials were designed with replicated blocks each treatment with a hydroponic NFT channel system with separate recirculated nutrients. An overview of the 25 experiments conducted over the project period is listed in Table 1.

Table 1. Trials established for VG04012

Trial Number	Dates (Location)	Treatments
1	April-May 2005 (Gosford)	Pathogenicity: 10 lettuce cultivars (Cvs) (Lansai, Concorde, EC123, Amadeus, Kipling, Levistro, Jamai, Kristine & Brown mignonette) x 2 seedling plug sizes x *Pythium*; *Phytophthora*; & *Pythium* plus *Phytophthora*
2	June-Aug. 2005 (Gosford)	Efficacy: 2 Cvs (Green oak & Red mignonette) x *Phytophthora* plus *Pythium* x disinfectants: calcium hypochlorite; chlorine dioxide; didecylidimethyl ammonium chloride (Sporekill™); iodine "x 1 unit only" and monochloramine (Pythoff®).
3	Oct.-Nov. 2005 (Gosford)	Efficacy: 4 Cvs (Green oak, Red oak, Red coral & Red mignonette) x *Phytophthora* plus *Pythium* x microbial biocontrols (*Bacillus subtilis* formulated as Fulzyme™ Plus [*FZ Plus*]); *Trichoderma spp.* formulated as Tri-D-25®*, Pseudomonas putida* (isolated from healthy lettuce roots); and a combination of *Bacillus* and *P. putida*
4	Dec. 2005 (Gosford)	Efficacy: 4 Cvs (Green oak, Red oak, Red coral & Red mignonette) x *Phytophthora* plus *Pythium* x treatments: disinfectant (Sporekill™); a non-ionic surfactant Agral®; and growth promoting product, Hygrozyme®)
5	Feb.- March	Efficacy: 2 Cvs (Kristine & Anikai) x *Pythium* x

Trial Number	Dates (Location)	Treatments
	2006 (Gosford)	treatments: microbial biocontrol (*B. subtilis* [*FZ Plus*]); a growth promoter (Hygrozyme®); and the fungicide propamocarb (Previcur®)
6	April-May 2006 (Gosford)	Pathogenicity: 1 Cv. (Red oak) x 9 *Pythium* isolates x 4 temperature treatments, ambient to 30-40^{o}C
7	Sep.-Nov. 2006 (Gosford)	Pathogenicity: 2 Cvs (Green sun & Ember) x 2 *Pythium* species x 2 inoculum rates
8	Nov.-Dec. 2006 (Gosford)	Pathogenicity: 2 Cvs (Fabietto & Ember) x 2 *Pythium* species x 5 treatments (using roots from previous trial as inoculum)
9	Dec. 06 – Jan. 07 (EMAI)	Pathogenicity: 1 Cv. (Ember) x 4 pathogens (*Pythium, Phytophthora, Thielaviopsis* + pathogen combination)
10	Jan.-Feb. 2007 (EMAI)	Pathogenicity: 2 Cvs (Murai & Ember) x 4 pathogens (*Pythium, Phytophthora* and *Thielaviopsis* + pathogen combination)
11	Mar.-Apr. 2007 (Gosford)	Pathogenicity: 1 Cv. (brown mignonette) X 9 isolates: 3 *Phytophthora*; 3 *Pythium*, 1 *Thielaviopsis basicola* & 2 *Ceratocystis paradoxa*
12	Mar.-Apr. 2007 (EMAI)	Efficacy: 1 Cv. (brown mignonette) +/- *Phytophthora* inoculum x microbial biocontrol *FZ Plus* & Agral® x +/- heating of nutrient solution
13		Iodine disinfection laboratory assays
14	Apr.-June 2007 (EMAI shadehouse)	Pathogenicity in seedlings: 1 Cv. (brown mignonette) x *Fusarium* (isolate 07/178-1)
15	May-June 2007 (EMAI)	Efficacy: 1 Cv. (Murai) x *Phytophthora* x microbial biocontrols (*B. subtilis* formulated into *FZ Plus* & Companion®)
16	June-July 2007 (EMAI)	Efficacy: 1 Cv. (Murai) x *Phytophthora* x microbial biocontrols (*FZ Plus* & *Streptomyces lycius* formulated in Microplus^{TM}) x (+/- root moisture stress)
17	July-Aug. 2007 (Gosford)	Pathogenicity: 1 Cv. (Red Ferrari) x 3 *Phytophthora* isolates; 3 *Pythium* isolates, 1 *Thielaviopsis basicola* isolate & 2 *Ceratocystis paradoxa* isolates
18	Aug.-Sept. 2007 (EMAI)	Efficacy: 1 Cv. (Red Ferrari) x *Phytophthora* x microbial biocontrols (*FZ Plus* & Superzyme®) x (+/- root moisture stress)
19	Sept.-Oct. 2007 (EMAI)	Pathogenicity: 1 Cv. (Murai) x 11 *Pythium* isolates x 4 reps.
20	2007 (EMAI)	Efficacy: Sonication & Ultra-violet (UV) disinfection laboratory assays
21	Sept.-Oct. 2007 (Gosford)	Pathogenicity: 4 Cvs (Amadeus, Kidance, Anikai & Nation) +/- *Phytophthora*
22	Oct.-Nov. 2007	Efficacy: 2 Cvs (Murai & Red Ferrari) x

Trial Number	Dates (Location)	Treatments
	(EMAI)	*Phytophthora* x disinfectants (Sporekill™ & Pythoff®) x 2 dilution rates x Electro Units (1&2)
23	Nov.-Dec. 2007 (EMAI)	Efficacy: 2 Cvs (Murai & Jamai) x microbial biocontrols *FZ Plus* x 4 application rates & seedling drench
24	Jan.-Feb. 2008 (EMAI)	Efficacy: 2 Cvs (Aniki & Nation) x 2 disinfectants (Sporekill™ & Pythoff®) x 2 dilution rates
25	Mar.-Apr. 2008 (EMAI)	Efficacy: 1 Cv. (Murai) +/- *Phytophthora* x disinfectants (Sporekill™ & Pythoff®) x re-applications

Pathogens were grown on potato carrot agar (PCA) at 25^{o}C for 10-14 days. Cultures were homogenised in distilled water and an equivalent of 1-2 plates were added to specified treatment tanks. Serial dilution of the inoculum suspension and culturing to agar media was used to estimate pathogen concentrations. Non-colonised PCA plates where homogenised to serve a negative control treatments.

Seeds were grown by a commercial seedling producer in plugs (198/tray) and were transplanted to NFT channels and allowed to establish prior to treatment applications. Samples were taken from seedlings upon receipt and roots were screened for background or confounding plant pathogens as described above in section 1.2.

Tanks were topped up with fresh nutrient as required. The plants were grown to maturity and harvested. Whole plants were drained free of water and weighed to obtain total wet weights. Data was analysed using ANOVA and treatment differences were tested using Fishers protected LSD test to compare treatment differences (LSD) test at 5% level.

Roots were sampled and cultured as described above (section 1.2) to determine pathogen colonisation.

2.1 Pathogenicity Trials

Pythium, *Phytophthora, Fusarium* and *Thielaviopsis* isolates were tested in a number of replicated trials (Table 1 above).

The effect of warmer root-zone temperatures was tested in some trials at the EMAI site. Aquarium heaters were used in the nutrient tanks that raised temperatures by 3-4^{o}C above unheated tanks. A separate trial at Gosford (#6, Table 1) simulated root-zone temperature effects in the presence and absence of plant pathogens. Plants were removed from the nutrient tanks and placed in plastic bags and their roots were emersed in water baths set at ambient, 30, 35, and 40^{o}C. Plants were returned to their respective channels after 2 hours of heat treatments.

The effect of root-zone moisture stress was induced in other trials (Table 1). Turning off pumps and allowing nutrients to flow back into respective tanks achieved this. Shutdowns were carried out in the daytime and on 1-3 occasions of weekly treatments within an experiment for varying time periods (4-7 hours each). Plants were monitored and systems were restarted when plants had wilted.

Individual trials are detailed below:

2.1.1 Trial #10: Isolates of *Pythium*, *Phytophthora*, and *Thielaviopsis* where screened for pathogenicity to 2 lettuce cultivars (Murai and Ember). Isolates where grown on selective agar and homogenised with a volume of sterile water. One plate of inoculum was added per 20lt tank. Treatments were replicated 10 times. Plants where grown to maturity and wet weights where recorded. Analysis of the data was determined by pairwise T tests with unequal variances (an LSD is not applicable). Pairwise comparisons are only shown if the omnibus ANOVA F Test to test the null hypothesis of no treatment (isolate) effects (allowing for replicate effects) is significant.

2.1.2 Trial #11: A pathogenicity screening trial for 3 *Pythium* isolates, 3 *Phytophthora* isolates, 1 *Thielaviopsis* isolate and 2 *Ceratocystis* isolates to the lettuce cultivar, brown mignonette. There ware a total of 10 treatments with 4 replications. Plants where grown until mature and wet weights were recorded.

2.2 Disinfection Efficacy trials

Several trials determined efficacy of the available disinfection options for root diseases caused by *Pythium*, *Phytophthora* and *Thielaviopsis*. They are detailed below, and included disinfectants, a surfactant and two frequency units.

2.2.1 Iodine

Iodine *in vitro* assays were performed at the Plant Health Research laboratory, EMAI. Mycelial isolates (colonised cloth squares) of the pathogens *Phytophthora drechsleri*, *Pythium aphanidermatum*, *Fusarium oxysporum* and

Thielaviopsis basicola were dipped or in varying concentrations of iodine (0,1, 5 &10mL/1000L) for time periods (1,2, 5 & 10 minutes). Spore suspensions of *Pythium aphanidermatum* and *Fusarium oxysporum* were similarly exposed to varying concentrations of iodine (0,2,5,10 & 20mL/1000L) for time periods (5, 10, 20 & 30 minutes).

An evaluation of a commercial iodine dosing system (the I-San™ system distributed by Ioteq Australia Pty Ltd). was conducted at the NSW DPI Centre for Greenhouse Horticulture, Gosford. This system was originally developed as a post-harvest treatment for fruit and vegetables. The unit incorporates an anion-exchange resin chamber to filter iodine from the return nutrient solution that avoids a build-up of undesirable iodide complex molecules.

2.2.2 Calcium hypochlorite and Chlorine dioxide (ClO_2)

Both chemicals were applied to hydroponic nutrient tanks in Trial #2 (Table 1).

An assessment was made on a commercial ClO_2 unit (Grayson Australia – Tecnica Pty Ltd) that activates and injects directly into the nutrient supply line. The output concentration of ClO_2 was measured using a commercial meter. Laboratory assays were conducted on the treated nutrient for the plant pathogens *Pythium* spp. and *Fusarium* by the methods described below. *Pythium* spp. were baited from 500mL water samples by placing 5 agar blocks (20x20x5mm potato carrot agar containing the antibiotics pimaricin @ 10ppm and rificamicin @10ppm [PCA-PR]) for 12 hours before retrieving them with forceps, plating them onto PCA-PR agar plates and incubating them for 3 days at 25^{o}C. Plates were periodically examined under a light microscope (100x magnification) growth of typical *Pythium* mycelium. Sub-cultures were made to confirm and characterise *Pythium* species. *Fusarium* spp. were detected by passing a 500mL sample of treated nutrient through a filter funnel lined with filter paper (Whatman No1). The filter paper was then placed on agar plates (1/4-strength potato dextrose containing the antibiotic novobiocin @ 100ppm) and incubated for 3 days at 25^{o}C. Plates were periodically examined under a light microscope (100x magnification) growth of typical *Fusarium* mycelium and microconidia. Sub-cultures were made to confirm and characterise *Fusarium* species.

2.2.3 The quaternary ammonium disinfectant, Sporekill™ (didecylidimethyl ammonium chloride)

Sporekill™ was tested at 10, 20 and 170mL/1000L in trials #2, #22, #24 and #25(Table 1). In the later trials (#24 & #25), Sporekill™ was added at 10 and 20ppm before adding *Phytophthora* and *Pythium*, and then also after the inoculum was added.

2.2.4 The non-ionic wetting agent, Agral®

This product was assessed in trials #4 and #12 (Table 1) at two application rates (15 & 20mL/1000L).

2.2.5 Monochloramine, PythOff®

This product was tested used in trials #2, #22, #24 and #25 (Table 1). It was applied to nutrient tanks at 10-80ml\1000L before or after *Phytophthora* and/or *Pythium* inoculum was added.

2.2.6 Sonication & Ultra-violet (UV) disinfection

An assessment of a combined UV and sonication unit (Omni Environmental Pty Ltd) was conducted for its efficacy to the same isolates of *Fusarium*, *Pythium* and *Thielaviopsis* that were used in the iodine assays above. Two flow rates were assessed (60 and 300mL/s). The test was repeated three times and means of colony forming units (cfu) of the organisms were calculated before and after water treatments.

A separate UV unit was assessed on-farm to disinfect water of plant pathogens, *Pythium*, *Phytophthora* and *Fusarium*. Samples of water (1-2L), pre- and post-UV treatment were assayed for the presence of plant pathogens. The sampling and testing period was over 3 months between August and November 2006. The first sets of water samples were used to refine procedures for sampling and laboratory detection. *Pythium* and *Phytophthora* were detected using a baiting technique with semi-selective agar media. *Fusarium* was detected from a concentrated filtrate of 1L of water samples that was then placed on selective and general-purpose agar media. These methods are largely qualitative, but comparing recovery rates gives a crude estimate of relative efficacy of the UV treatments. Quantitative estimates of *Pythium* and *Fusarium* in water samples were also made using dilution end-point assays.

During November 2006 an experiment was conducted to quantify the efficacy of the UV unit to *Fusarium* and *Pythium*. It was decided to inoculate the water holding tank with spores of the *Fusarium* fungus since endogenous levels were below detectable limits in previous trials. To inoculate *Fusarium* into the tank, 25 cucumber stems were selected from the farm. These cucumber stems were highly infested with *Fusarium*. Stems were covered with sporodochia (fungal structures producing orange-coloured spore masses). After the stems were washed to release the *Fusarium* spores, the water containing those spores was added to the tank and mixed in with a plastic pipe.

Water samples were taken soon after fungal spores were added to the tank. Another two water samples were taken before the UV light was turned on. Once the UV light was turned on water samples were taken after 10 and 20 mins. The UV light was then turned off, and a water sample was taken after 10 mins. The UV light was again turned on and samples were taken after 10 and 20 mins. A final water sample was taken 10 mins after the UV unit was turned off for the final time. The purpose of the additional water samples was to see if the *Fusarium* and *Pythium* levels had changed following the tank water flow.

A further set of water samples was taken from the same tank a week later (14/11/06). On the 22/11/06 more water samples were taken and, to reduce

the amount of *Fusarium* present in the tank, half the water was drained and refilled with runoff water. Water samples were taken 5 and 10 mins before the UV unit was turned on and then 10 mins after the UV unit had been on.

2.3 Microbial biocontrol & growth stimulant efficacy trials

Products evaluated in trials are listed in Table 2 below.

Table 2. Microbial biocontrols & growth stimulants tested

Product	Active ingredients	Rate/use-pattern	Assessment trial #
Hygrozyme®	Unknown chemicals	1L/1000L	5,
Fulzyme™ Plus *(FZ plus*) JH Biotech Inc (Zadco For Quality Gro Ltd)	*Bacillus subtilis*	2L/1000L	3, 4, 5, 12, 15, 16, 18, 23
Companion® Spray Grow Ltd	*Bacillus subtilis*	2.5L/1000L	15
Microplus™ Organic Farming Systems Ltd	*Streptomyces lycius*	0.4kg/10000L	16
Lab isolate #06/966	*Pseudomonas putida*	75ml / 20L	16
Superzyme® JH Biotech Inc (Zadco For Quality Gro Ltd)	*Bacillus subtilis* & *Pseudomonas putida*	3kg/1000L	3
Tri-D-25® JH Biotech Inc (Zadco For Quality Gro Ltd)	*Trichoderma harzianun & T. koningii*	3g / 1L	3
Previcur® Bayer	propamocarb	@ 1.5L/1000L	5

Individual trials are detailed below:

2.3.1 Trial #3: Microbial biocontrol products and *Phytophthora drechsleri* on four hydroponic lettuce cultivars

Four cultivars of lettuce where obtained from a commercial seedling producer: Red Oak; Green Oak; Red Coral and Red Mignonette. They were randomly assigned to positions along channels and planted on 6th October 2005. Each channel contained forty plants (10 x 4 cultivars), plus buffer plants at each end.

The trial design consisted of five treatments: the three microbial inoculants with *Phytophthora drechsleri* (PHDS collection # 04/262); a positive control for *Phytophthora*; and a negative control. These five treatments were randomly assigned to channels in seven replicated blocks.

Plants were grown until maturity and harvested on the 10th November 2005. Tanks were topped up with water and complete nutrients as required. Whole plants were drained free of water and weighed to obtain wet weights. Sub-samples of roots were taken and cultured to agar media to determine their *Phytophthora* colonisation.

Plant wet weights were analysed using an analysis of variance with a split plot design on the following model: Weight = Treatment + *Channels* + Cultivar + Treatment x Cultivar + *Channel x Cultivar* + *Error*. The terms were assumed to have random effects and the error follows a normal distribution. Treatments x cultivar means were compared using the Least Significant Difference (LSD) test at the 5% level.

2.3.2 Trial #5: Microbial inoculant *FZ Plus* and *Pythium spp.*

The trial design consisted of five treatments: a microbial inoculant (*FZ plus* @ 2ml/L); propamocarb (Previcur® @ 1.5ml/L) drenched on seedlings prior to transplanting to the channels; a growth enhancer (Hygrozyme® at the recommended rate in appropriate tanks); a negative control; and a positive *Pythium* inoculum control. All tanks except for the negative controls received *Pythium* inoculum. These five treatments were randomly assigned to channels in seven replicated blocks.

Pythium inoculum was applied to appropriate nutrient tanks seven days after the microbial inoculant and plant growth enhancer.

Wet weight data were fitted into the following model:

Weight = fixed (treatment + cultivar + [treatment x cultivar]) + random (block + channel + [channel x cultivar] + error.

All parameters were estimated using the residual maximum likelihood (REML) estimation and the analysis was run on Genstat (VSN International 2003)

2.3.3 Trial# 12: Microbial inoculants *FZ Plus* verses *Phytophthora* on 1 lettuce cultivar (Brown mignonette) with and without heating of nutrient solution.

The trial design consisted of 4 treatments of which 3 were heated by placing an aquarium heater into the nutrient tank. These treatments were: *FZ plus* with *Phytophthora*, a positive control for *Phytophthora*, a nil inoculum control and a further *Phytophthora* treatment with no heat.

The 4 treatments were randomly assigned to channels in 10 replicated blocks. Plants were grown until maturity and harvested on the 20th April 2007. Tanks were topped up with nutrients as required. Whole plants were drained free of water and weighed to obtain wet weights.

Data was analysed using ANOVA and treatment differences were tested using a least significance difference (LSD) test at 5% level.

2.3.4 Trial #16: Efficacy of *FZ Plus* and a commercial microbial product containing a *Strepyomyces* sp. for *Phytophthora* in Hydroponic lettuce.

Seeds of the cultivar, *Murai* (Rijk Zwaan Seeds) were grown. All Nutrient tanks where heated with aquarium heaters. Heated nutrient temperatures measured in the channels ranged between 10- 29^{o}C. *Phytophthora cryptogea* (PHDS isolate collection 06/966/2) was used. A randomised complete block design was used consisting of 6 treatments that were replicated 8 times.

The water was shut down on 3 occasions for 8 hours each to increase plant stresses and therefore disease expression. Data was analysed using ANOVA and treatment differences were tested using a least significance difference (lsd) test at 5% level. Fresh weight data were fitted with a linear model with a spatial correlation (first order autocorrelation) between channels to account for environmental effects if present. A residual maximum likelihood (REML) technique was used to estimate all parameters and the analysis was run on Asreml Window version 2 (Gilmour *et al.*, 2006).

2.3.5 Trial #18: Efficacy of *FZ Plus* and Superzyme® to *Phytophthora* on the cultivar Red ferrari, with and without heating of nutrient solution and root moisture stress.

The trial design consisted of 8 treatments in 6 randomised repetitions, consisting of negative and positive controls for pathogen inoculum, a negative pathogen inoculum control for the microbial biocontrol *FZ Plus*, and *Phytophthora cryptogea* (PHDS isolate collection 06/966/2) inoculum with *FZ Plus*. These treatments were duplicated. One of these was subjected to pump shutdown to induce water stress while the other had continuous flow. Water was heated to increase nutrient temperatures.

Plants were grown until maturity. Whole plants were drained free of water and weighed to obtain wet weights. Tanks were topped up with nutrients as required.

An analysis of variance (ANOVA) for a randomised complete block design (RCBD) was performed. Fisher's protected least significant difference (LSD) was used to compare treatment means.

Results & Discussion

1. Farm surveys & laboratory diagnosis

Surveys of 14 farms in NSW, two each in South Australia and Queensland and an enterprise in Victoria have determined very high root infection rates with the water moulds, *Pythium* and/or *Phytophthora*. In the cooler months these organisms caused sub-clinical infections (without discernable root injury or reduced plant growth). However, during the summer period root damage was increased with associated plant losses. Farms in all four states suffered significant losses with whole plantings discarded, while others only suffered minor losses. The one exception to this seasonal trend was the Victorian farm where losses were most severe in the cooler months.

Two *Pythium* species were isolated from affected plants. *Pythium polymastum* was identified from seedlings and plants from several farms in NSW and Victoria. This water mould has been previously recorded on lettuce in the USA (Drechsler, 1939) and Europe (Plaats-Niterink, 1975). It was not shown to be a significant pathogen. ITS sequences confirmed these fungal species names while the *Pythium* isolates clustered with GenBank database accessions of *P. latarium*, *P. dissotocum, P. diclinum, P. pachycaule* and *P. coloratum*. The latter name is used here, based upon characteristic morphological traits described for this species: a lilac oospore wall and a particular arrangement of sexual structures (antheridial stalks that encircle the oogonium). Both features were visible in water mounts examined by light microscopy (100-400x magnification). It would appear that the ITS does not discriminate this group of species. This result is consistent with other studies (Levesque & De Cock, 2004; Alhussaen, 2006). One isolate of *Phytophthora* (#06-948-1) clustered with *P. erythtoseptica,* while others clustered with *P. cryptogea* and *P. drechsleri*. Hutton and Forsberg (1991) have previously recorded a *Phytophthora* sp. associated with hydroponic lettuce root rot in Queensland.

Other potential plant pathogenic fungi were isolated from several NSW farms. *Thielaviopsis basicola* and a related fungus, *Ceratocystis* (anomorph *Thielaviopsis*) *paradoxa* were isolated from 2 farms. *T. basicola* is the cause of the disease, black root rot that affects a wide range of agricultural crops. A previous study in Australia has recorded *T. basicola* on hydroponic lettuce in Queensland (O'Brien & Davis, 1994). That study determined that peat material used for seedling production was a source of this fungus. *Rhizoctonia* spp. were rarely isolated but was associated with one incidence of large root disease losses in North Queensland in 2008. This isolate is yet to be characterised as it was detected near the conclusion of this project. *Fusarium oxysporum* was commonly isolated from all farms but was not associated with any wilt symptoms suggesting that it is not the lettuce wilt pathogen (*F. oxysporum* f.sp. *lactucae*).

A number of factors were identified as contributing to disease incidence and severity through the survey period. They are listed and discussed below:

- High nutrient temperature was the dominant factor associated with disease expression. Temperatures were logged at several sites exceeding 35^{o}C in channels during the day. These temperatures have been previously been shown to cause direct damage to roots even in the absence of plant pathogens (Tesoriero & Cresswell, 1995; and Alhassaen, 2006).

- Moisture stress correlated with increased incidence and severity of root diseases. Two causes of moisture stress were mechanical breakdowns or where some growers did not pump nutrient solution continuously. They timed pulses of nutrient even during the summer months.

- Some lettuce cultivars were observed to be more susceptible to disease than others, although several different cultivars were affected in some instances. In general, red/brown cultivars were associated with greater disease expression than green cultivars.

- There was a strong association between root disease and infection plant with Tomato spotted wilt virus (TSWV). Plants infected by TSWV eventually wilt and their roots blacken and rot with associated infections of *Pythium* and/or *Phytophthora* spp. One case demonstrated *Phytophthora* infection in such a TSWV-infected plant, while the neighbouring healthy plant remained free of root infection. Failure to remove and dispose TSWV infected plants therefore could act to encourage reservoirs for root rot pathogens, along with TSWV and its thrips vectors.

- Poor hygiene and crop management practices were common on certain farms where disease levels and associated losses were higher. Examples of such practices are: discarding diseased plants on the ground under the channels; poor seedling production hygiene leading to early infection of plants; and growing plants of different stages of maturity in the one system thereby allowing younger plants to be infected from the older ones.

When major disease problems were encountered, most growers attempted to clean out and sanitise the nutrient tank and channels with a disinfectant solution. Unfortunately, with poor hygiene practices (noted above) these efforts were of limited and temporary success.

2. Pathogenicity Trials

Pathogenicity was not demonstrated in all trials. In two cases inoculum failure was likely to have led to no disease expression but in several instances (particularly at the Gosford site) infection established in roots but no clinical symptoms nor significant growth retardation was observed. In such cases it was concluded that there was insufficient inoculum and plant stresses to induce pathogenesis. Listed below are individual trial results where pathogenicity was demonstrated.

2.1.1 Trial #10:

All isolates produced a significant yield loss compared to the Nil controls in both lettuce cultivars. Note (Table 3) Cv. Murai was affected more by the inocula than the Cv. Ember.

Table 3. Mean lettuce wet weights from pathogen treatments

Treatment	Isolate #	Mean wet weight cv. Ember	Mean wet weight cv. Murai
Nil control	-	264 d	226d
Pythium	06/706	247 cd	209c
Phytophthora	06/966	76 a	25a
Thielaviopsis	06/823	231 c	209c
Pythium,Phytophthora Thielaviopsis	06/706, 06/966, 06/823	167 b	65b

2.1.2 Trial #11:

All test isolates reduced mean weights of lettuce cv. *Brown mignonette* (Table 1). Only one of the 3 *Pythium* isolates significantly reduced growth, whereas all three *Phytophthora* isolates reduced mean weights by 38-46%.

The *Phytophthora* inoculum concentration was estimated to be 10^6 colony-forming-units per 100-litre tank.

Table 4. Mean lettuce wet weights from pathogen treatments

Treatment	Isolate #	Mean wet weight
Nil control	-	121a
Pythium colorarum	DAR77477	69 bc
Pythium coloratum	06-706	100 ab
Pythium coloratum	06-754-1	99 abc
Phytophthora erythroseptica	06-948-1	74 bc
Phytophthora cryptogea	06-919-2	64 bc
Phytophthora cryptogea	06-966-2	67bc
Thielaviopsis basicola	06-512	91 abc
Ceratocystis paradoxa	06-825	89 abc
Ceratocystis paradoxa	06-1003-1	96 abc
LSD at 5%		35

2.2 Disinfection Efficacy Trials

2.2.1 Iodine

Iodine at concentrations up to 10ppm (10mL/1000L) and exposure times up to 10 minutes was ineffective at killing the pathogens *Phytophthora drechsleri*, *Pythium aphanidermatum*, *Fusarium oxysporum* and *Thielaviopsis basicola in vitro* assays with mycelial isolates on colonised cloth squares. In spore

suspensions assays, the lethal dose for 100% kill (LD_{100}) for *Pythium aphanidermatum* was 30 minutes exposure at 5mL/1000L iodine or 5 minutes exposure at 10mL/1000L. For *Fusarium oxysporum* the LD_{100} was 30 minutes exposure at 10mL/1000L iodine or 5 minutes exposure at 20mL/1000L iodine. Some variability was experienced in these latter assays and lower concentrations such as 5 minutes exposure at 10mL/1000L was an LD_{100} despite growth occurring at longer exposure times.
Phytotoxicity (stunting and discoloration of roots) to hydroponic lettuce was experienced with the commercial iodine dosing system unit when iodine was applied at 5 and 10mL/1000L.

2.2.2 Calcium Hypochlorite and Chlorine dioxide (ClO_2)

Both chemicals were phytotoxic to lettuce when applied to hydroponic nutrient tanks, causing severe root damage and subsequent wilting (and death). Both products eliminated *Phytophthora* from the nutrient system. A review of this experiment determined that both chemicals were applied at rates higher than planned due to faulty kits (commercial dipsticks) used for chemical concentration estimates. Previous experience with potassium hypochlorite has indicated that lower concentrations (<5mL/1000L) are not phytotoxic. It does demonstrate that these disinfectants are potentially phytotoxic and that application errors can easily result in worse losses that those caused by plant pathogens. ClO_2 application is further complicated by the fact that it needs to be activated by an acid solution prior to addition to the nutrient solution. Activation (release of ClO_2) is dependent on several factors including temperature, pH and time.
The commercial ClO_2 unit had an output concentration of ClO_2 measured at 0.3ppm. Assays for plant pathogens in the treated nutrient solution determined the presence of *Pythium* and *Fusarium*, suggesting that this ClO_2 concentration is too low to be efficacious. The unit tested retails for about $6,000, which is unlikely to be economical for enterprises that have several nutrient tanks requiring a separate unit for each.

2.2.3 The quaternary ammonium disinfectant, Sporekill™

The higher concentration 170mL/1000L almost eliminated *Phytophthora* from the nutrient solution but was phytotoxic. The lower application rate also significantly reduced plant wet weights compared with untreated controls. *Phytophthora* and *Pythium* were still detected in root systems suggesting that this product has no total curative effect. Sporekill® failed to significantly control Phytophthora root rot and was phytotoxic at 10mL/1000L (Table 5).

Table 5. Lettuce wet weights for Trial #25

Treatment	Lettuce wet weight (g)
Nil inoculum	132.9 c
Phytophthora	77.4 a
Nil inoculum + Pythoff®	115.8 b
Phytophthora + Pythoff® (before)	83.1 a
Phytophthora + Pythoff® (after)	77.0 a
Nil inoculum + Sporekill®	111.2 b
Phytophthora + Sporekill® (before)	95.0 a
Phytophthora + Sporekill® (after)	106.9 ab

2.2.4 The non-ionic wetting agent, Agral®

This product was ineffective in reducing disease symptoms and infections at both application rates (15 & 20mL/1000L).

2.2.5 Monochloramine, PythOff®

This product was tested at 10-80mL/1000L and was ineffective at reducing *Phytophthora* from the nutrient system or from lettuce roots (Table 5). It was also phytotoxic at 10 mL/100L.

2.2.6 Sonication & Ultra-violet (UV) disinfection

The combined UV and sonication unit was 100% efficacious for all three pathogens at a flow rate of 60mL/s while the faster flow rate eradicated *Pythium* and *Fusarium*, and was 97% effective for *Thielaviopsis*. This unit retails for $3,500 and would be useful for farms with only 1 or two recirculating units but of marginal value for larger enterprises that have several nutrient tanks requiring a separate unit for each.

The 'on-farm' UV unit was shown to be efficacious for *Pythium* and *Fusarium* with a flow rate of 1L/s. The results of all the preliminary assays are presented in Table 6. *Pythium* was consistently recovered from untreated water in the holding tank. The recovery rate from these water samples was highly variable, averaging about half that of water taken from the waste sump. The UV treatment effectively eliminated *Pythium* on all six sampling dates. This unit retails for $9,000, again requiring a careful benefit/cost analysis.

Table 6. Recovery of plant pathogens from agar baits or colony-forming units (c.f.u.) on filter paper incubated on PPA medium

Sample Date (PHDS#)	Sample origin /treatment	*Pythium*	*Phytophthora*	Other fungi*
23/08/06 (06/637)	Holding tank	3/10	n.d.	n.t.
	UV	0/10	n.d.	n.t.
5/09/06 (06/678)	Waste sump	10/10	n.d.	n.t.
	Holding tank	10/10	n.d.	n.t.
	UV	0/10	n.d.	n.t.
8/09/06 (06/686)	Holding tank	7/10	n.d.	>100 c.f.u.
	UV	0/10	n.d.	46 c.f.u.
	ClO_2	7/10	n.d.	n.t.
13/09/06 (06/699)	Holding tank	8/10	n.d.	>10 c.f.u.
	UV	0/10	n.d.	>10 c.f.u.
14/09/06 (06/703)	Holding tank	8/10	n.d.	n.t.
	ClO_2 (0.3ppm)	8/10	n.d.	n.t.
20/09/06 (06/721)	Waste sump	10/10	n.d.	>10 c.f.u.
	Holding tank (top)	10/10	n.d.	>10 c.f.u.
	Holding tank (bottom)	10/10	n.d.	>10 c.f.u.
	UV@ 10min	0/10	n.d.	2 c.f.u.
	UV@ 20min	0/10	n.d.	2 c.f.u.
4/10/06	Holding tank	2/10	n.d.	>10 c.f.u.

Sample Date (PHDS#)	Sample origin /treatment	*Pythium*	*Phytophthora*	Other fungi*
(06/760)	UV	0/10	n.d.	5 c.f.u.
2/11/06	Waste Sump	10/10	n.d.	n.d.
	Holding tank	1/10	n.d.	n.d.

n.d. = not detected; n.t. = not tested
c.f.u. = direct determination of fungal colony forming units/plate
Only trace levels of *Fusarium* were confirmed from these assays, and only from the waste sump and holding tank. Most fungal isolates counted were identified as the saprophyte, *Geotrichum*.

The data from the *Fusarium* inoculation trial is presented in Tables 7, 8 and 9.

Table 7. Recovery of *Fusarium* and *Pythium* from water samples taken on the day of inoculation (8/11/06)

Water sample	Pathogen	
	Fusarium	**Pythium**
Pre UV ~3 mins post inoculation of tank	+++	+
Pre UV ~5 mins post inoculation of tank	+++	+
Pre UV ~10 mins post inoculation of tank	+++	+
UV on for 10 mins (first run)	++	n.d.
UV on for 20 mins (first run)	++	n.d.
UV off water has run for 10 mins	+++	+
UV on for 10 mins (second run)	+	n.d.
UV on for 20 mins (second run)	+	n.d.
UV off for 10 mins	+++	+

+++ = high level of pathogen; ++ = moderate level of pathogen; + = low level of pathogen; n.d. = not detected

The UV treatment reduced *Pythium* to undetectable levels and significantly reduced *Fusarium* levels. The filter paper used in this assay turned pink with the development of *Fusarium* much more quickly from untreated water samples. The level of inoculum put into the tank was possibly too high.

Table 8. Recovery of *Fusarium* and *Pythium* from water samples (14/11/06)

Water sample	Pathogen	
	Fusarium	***Pythium***
Pre-run 5 mins	+	+
Pre-run 10 mins	5120 cfu/mL	+
UV on for 10 mins	80 cfu/mL	n.d.

c.f.u. = most probable number determination of fungal colony forming units

When the water was re-sampled six days later the UV unit eradicated the *Pythium* from the water as previously. The UV treatment reduced the *Fusarium* levels by 98%, however it did not eradicate it (Table 8). *Fusarium* levels were again very high (>5,000 c.f.u./mL) and unlikely to be seen in a natural glasshouse recycled water situation.

After the tank water had been diluted *Fusarium* was no longer detectable from the UV treated water samples (Table 9). *Fusarium* levels in untreated water were about an order of magnitude less than was detected a week earlier, but still over 500 c.f.u./mL.

Table 9. Recovery of *Fusarium* and from water samples (22/11/06)

Water sample	*Fusarium* level
Pre-run 10 mins	~640 c.f.u/mL
UV on for 10 mins	n.d.
Post run UV off 10 mins	~640 c.f.u./mL

c.f.u. = most probable number determination of fungal colony forming units; n.d. = not detected

The UV unit is sufficient for eradicating *Pythium* from the tank water. The UV unit significantly reduces *Fusarium* in the water, and at lower initial concentrations, to below detectable limits. It would appear that the *Phytophthora* detected in the wastewater sump did not survive in the holding tank. Similarly, *Fusarium* was only found at trace levels in the waste sump and holding tank. Overall there appears to be less *Phytophthora* and *Fusarium* in the waste sump than there was when we previously assessed the efficacy of the slow sand filter. It was not clear whether this was due to residual chlorine dioxide (subsequently installed on the town water supply) or to other undetermined factors. Alternatively, chlorine dioxide treated recycled water (measured at the dripper as 0.3ppm) had similar *Pythium* recovery levels to the untreated tank water (Table 6).

4. Microbial biocontrol & growth stimulant efficacy trials

Specific trial results are listed below:

2.3.1 Trial #3: *Phytophthora* inoculum had no significant effect on wet weights for any of the lettuce cultivars. Only slight root discoloration was observed on the *Phytophthora* inoculation treatment compared with the negative control, but not enough difference to make an objective assessment. A low level of infection was determined when root pieces were cultured to agar media. *FZ Plus* and Superzyme® had no significant effect on wet weights compared with the control treatments. Tri-D-25® appeared to have a negative effect on wet weights and plant growth (Table 10).

Table 10. Effect of microbial inoculants and *Phytophthora drechsleri* on wet weights of four lettuce cultivars

Variety	*FZ Plus* + Phyt.	Neg. control	Phyt. control	S'zyme + Phyt.	Tri-D25 + Phyt.	SED	LSD at 5%	Overall means
Green Oak	473.6_a^A	428.2_a^{AB}	426.4_a^{AB}	397.9_a^B	160.9_b^C	24.01	47.98	380.5_a
Red Coral	157.6_d^A	138.6_c^A	132.5_c^A	169.6_d^A	74.5_c^B	24.01	47.98	134.6_d
Red Min	296.9_c^{AB}	328.6_b^A	300.2_b^{AB}	280.1_c^{BC}	232.6_a^C	24.01	47.98	289.3_c
Red Oak	367.2_b^A	334.0_b^A	330.9_b^A	361.0_b^A	150.7_b^B	24.01	47.98	310.3_b
SED	17.97	16.49	16.49	17.97	17.97			7.78
LSD at 5%	35.76	34.32	34.32	35.76	35.76			15.49
Overall means	323.8^A	307.3^A	297.5^A	302.1^A	154.7^b	18.81	38.6	

Note: Letters in subscript denote significant differences between cultivars whereas the letters in superscript denote significant differences between treatments.

2.3.2 Trial #5: *FZ Plus* treated plants grew significantly bigger than those in all other treatments (Table 11). This growth stimulation was independent of *Pythium* inoculum, which had no significant effect on plant wet weights. Hygrozyme®, the growth stimulant with undisclosed active ingredients did not significantly influence plant growth compared with untreated controls. Pathogens were also detected in roots of treated systems. Anecdotal reports of stimulated growth were not substantiated in this limited study with this product. Further experimental data are required before any definitive assessment can be made for this product.

Table 11. Effect of biological and chemical treatments and *Pythium* on wet weights of two lettuce cultivars for Trial #5

	Cultivar		
Treatment	**Green**	**Red**	**Means***
FZ Plus	468.0	354.2	411.6a
Previcur ®	427.3	320.7	374.0b
Hygrozyme ®	439.7	323.7	381.7b
Neg. Control	407.7	314.4	361.0b
Pythium Control	410.8	313.7	362.2b
SED			12.5
LSD (5%)			25.9
Means*	429.8A	324.6B	

*Means with different letters indicates significant difference at 5% level

2.2.3 Trial #12: Plants growing with *Phytophthora* alone showed reduced growth and wilted slightly during the day. *Phytophthora* significantly reduced mean wet weights compared with uninoculated controls. Agral® had no significant effect on disease expression or yields. *FZ Plus* treated plants did not exhibit disease symptoms and yields were equivalent to uninoculated

controls. Yield from the unheated treatment was not significantly different from heating in the presence of *Phytophthora*.

Table 12. Lettuce (cv. *Brown Mignonette*) wet weights for Trial #12

Treatment	Means
Nil	194.24a
Phytophthora 06/966/2	170.33b
Phytophthora +Agral®	177.95b
Phytophthora + *FZ Plus*	194.54a
Phytophthora +No heat	181.06b
LSD	12.37

Means with different letters indicate significant difference at 5% level

2.3.4 Trial #15: Switching the water off significantly influenced disease severity as expressed in wet weights with *Phytophthora* infection in the overall results. In the five replicates (Reps 6-10) that experienced more even growing conditions, *FZ Plus* treated plants did not exhibit disease symptoms and yields were equivalent to uninoculated controls. Companion® did not provide protection and produced a softer leafed lettuce with colour change. This latter result could be explained by the fact that this product was not freshly purchased and had been stored in a refrigerator for 18 months that may have allowed undesirable contaminants to grow. It does highlight that microbial biocontrols may have a definable shelf life.

Nutrient temperatures measured in the channels ranged between 15-34^{o}C. A light intensity meter was used to take readings along the northern and southern sides of the growing structure. The light intensity was double on the northern side due to the sun dropping to its winter position. Nutrient and micro-climates also varied by 1 to 3 degrees Celsius from either side. Both these combinations contributed to varying grow rates that confounded wet weight data. Therefore a split analysis of yield data was carried out.

Data was grouped into three sets: Group 1 comprised of replicates 1 and 2; Group 2 comprised of replicates 3, 4 and 5; and Group 3 included replicates 6 to 10. A linear mixed model was fixes to the data with the following model:
WT= fixed (group + treatment + interaction) + random (replicate + channel + error)

A residual maximum likelihood (REML) technique was used to estimate all parameters and least significant difference test was used to compare treatment differences within each group.

Table 13. Mean wet weights of lettuce for Trial #15

	Group 1	Group 2	Group 3	Overall
Treatment	**Rep 1-2**	**Rep 3-5**	**Rep 6 – 10**	**Rep 1- 10**
Nil	78.67ab	57.50a	107.30ab	81.16ab
Phyto	47.63b	41.39a	101.52b	63.51c
Phyto + *FZ Plus*	70.71b	40.36a	125.52a	78.86abc
Phyto + Companion®	54.25b	42.11a	97.68b	64.68bc

Phyto + water on	112.25a	46.42a	108.00ab	88.89a
SED	16.95	13.84	10.72	8.12
LSD 5%	34.71	28.34	21.95	16.63

Means with different letters indicate significant difference at 5% level
There was a significant effect of treatment on the plant weight (P=0.025) and significant interaction between treatment and groups (P=0.033).

2.3.5 Trial #16: Even growth rates occurred across the structure. All plants wilted temporarily during water stress events. Plants growing with *Phytophthora* alone showed reduced growth and wilted slightly during the day. Mean wet weights were used as yield estimates and are listed in Table 14.

Table 14. Mean lettuce fresh weights for Trial #16

Isolate	Mean wet weight	SE
NIL	121.50a	6.21
Phytophthora	73.91c	6.19
Phytophthora + *FZ Plus*	127.95a	6.20
Phytophthora + *Streptomyces*	92.78b	6.21
NIL+ *FZ Plus*	130.57a	6.19
NIL+ *Streptomyces*	117.98a	6.20
SED	8.62	
LSD5%	17.24	

Means with different letters indicate significant difference at 5% level

FZ Plus treated plants did not exhibit disease symptoms and yields were equivalent to the uninoculated controls in the presence of *Phytophthora*. The treatment containing the *Streptomyces* sp. did provide some significant disease protection but not equivalent to the negative control of *FZ Plus* treatments (Table13). Neither product appeared to stimulate plant growth in the absence of *Phytophthora,* suggesting that their effect was as disease suppressants rather growth stimulants.

2.3.6 Trial #18: This trial successfully demonstrated the efficacy of the microbial biocontrol *Bacillus subtilis* (*FZ Plus*) to *Phytophthora cryptogea* (PHR isolate number 06/966-2) on the lettuce cultivar (*Red Ferrari*).

Table 15. Mean lettuce fresh weights for Trial #18

Treatment	Means
Nil (water on)	115.83c
Nil (water on / off)	93.33b
Phytophthora (water on)	41.00a
Phytophthora (water on / off)	39.33a
Nil + *FZ Plus* (water on)	119.33c
Nil + *FZ Plus* (water on / off)	95.67b
Phytophthora + *FZ Plus* (water on)	121.00c
Phytophthora + *FZ Plus* (water on / off)	97.33b

Disease symptoms were expressed in the *Pyhtophthora cryptogea* treatments equally, with or without moisture stress. Many of these plants were near permanent wilting and most of the weight recorded was the root plug. Moisture stress did significantly affect yields in the negative pathogen inoculum control and in the presence of both the pathogen and *FZ Plus*.
FZ Plus had no growth stimulatory effect in the absence of the pathogen inoculum.

References

Anonymous (2001). Hydroponics as an agricultural production system. A report for the Rural Industries Research & Development Corporation by Hassall & Associates Pty Ltd. RIRDC Publication No 01/141, 72pp.

Alhassaen, K. (2006). *Pythium* and *Phytophthora* associated with root disease of hydroponic lettuce. PhD thesis, University of Technology, Sydney, 328pp.

Altschul S., Gish W., Miller W., Myers E. and Lipman D.J. (1990). Basic local alignment search tool. Journal of Molecular Biology 215: 403-410.

Bentley S. and Bassam B.J. (1996). A robust DNA amplification fingerprinting system applied to analysis of genetic variation with *Fusarium oxysporum* f.sp. *cubense*. Journal of Phytopathology 144: 207-213.

Burgess LW., Summerell BA., Bullock S, Gott KP and Backhouse D (1994) *'Laboratory Manual for Fusarium Research'* (3rd Edition) University of Sydney, Sydney

Drechsler, C. (1939). Three species of *Pythium* with large oogonial protuberances. Phytopathology 29:1005-31.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Server. 41:95-98.

Howlett, B., Brownlee, A., Guest, D., Adcock, G. and McFadden, G. (1992). The 5S ribosomal RNA gene is linked to large and small subunit ribosomal RNA genes in the oomycetes, *Phytophthora vignae*, *P. cinnamomi*, *P. megasperma* f.sp. *glycinea* and *Saprolegnia ferax*. Current Genetics 22: 455-461.

Hutton, D.G. and Forsberg, L. (1991). Phytophthora root rot in hydroponically grown lettuce. Australasian Plant Pathology, 20:76-79.
Matuo, T. and Motohashi, S. (1967). On *Fusarium oxysporum* f.sp. *lactucae* n.f. causing wilt of lettuce. Transactions of the Mycological Society of Japan, 32:13-15.

Levesque, C.A. and de Cock, A.W.A.M. (2004). Molecular phylogeny and taxonomy of the genus *Pythium*. Mycological Research, 108:1363-1383.

O'Brien, R. and Davis, R. (1994) Lettuce black root rot – a disease caused by *Chalara elegans.* Australasian Plant Pathology, 23: 106-111.

Plaats-Niterink, A.J. van der (1975). Species of *Pythium* in the Netherlands. Netherlands Journal of Plant Pathology 81:22-37.

Plaats-Niterink, A.J. van der (1981). Monograph of the genus *Pythium*. Studies in Mycology 21:242pp.

Stanghellini, M.E. and Rasmussen, S.L. (1994). Hydroponics – A solution for zoosporic pathogens. Plant Disease 78:1129-38.

Tesoriero, L., Carrus, R., Bertus, F., Jarvis, J., Ghalayini, A. and Forsyth, L. (2007). Characterisation and pathogenicity of fungi associated with roots of hydroponic lettuce. Australasian Plant Pathology Society Conference, Adelaide - September, 2007.

Tesoriero, L., Forsyth, L. and Carrus, R. (2008). Biocontrol of Phytophthora Root Rot of lettuce growing in hydroponic systems, ANZBC Conference, Sydney in February 2008 (Abs.).

Tesoriero, L.A. and Cresswell, G. (1995). Disease-temperature interactions in NFT. In '*Proceedings of the 2nd* Australian Hydroponic Conference, Sydney, Australia' (Eds F. Biggs and T. Biggs) (Australian Hydroponic Association Inc.), 39-42.

Tesoriero, L.A., Cresswell, G. and Gillings, M. (1991). Management principles for reducing diseases in soilless (hydroponic) systems. In 'Proceedings of the First National Conference of the Australian Cociety of Horticultural Science'. Macquarie University, Sydney, Australia. (Australian Society of Horticultural Science).

White, T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 In: *PCR Protocols: A Guide to Methods and Applications*, Editors: Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White. Academic Press, Inc., New York.

Technology Transfer

Some highlights of technology transfer outputs and activities were:

- Presentation of project updates and root disease management strategies at grower meetings – NSW Farmers group (4 times/year at Kellyville, NSW)

- Collaborating individually with growers in NSW, Queensland, South Australia and Victoria who have participated in farm surveys for root rot diseases. Growers were given diagnostic laboratory reports on their crop's disease status and management options.

- An expanded and refereed abstract was accepted for Australasian Plant Pathology Society Conference in Adelaide September, 2007 (Tesoriero *et al*., 2007). Mr Tesoriero presented this paper at the conference. The title was: *Characterisation and pathogenicity of fungi associated with roots of hydroponic lettuce*.

- A presentation and abstract entitled: *Biocontrol of Phytophthora Root Rot of lettuce growing in hydroponic systems*, for the ANZBC Conference, Sydney in February 2008.

- An A1-sized poster published on common lettuce diseases.

- NSW DPI held an hydroponic lettuce conference in June 2006 at Richmond, NSW. Mr Tesoriero presented a paper (included in the conference proceedings) on root disease management.

- An industry meeting for hydroponic lettuce growers was also held at the University of Western Sydney, Richmond 23rd February 2007 where Mr Tesoriero presented information on root disease management.

- A full day workshop was held for lettuce IPM in November 2007 where root disease management guidelines were circulated.

- A 'Lettuce Leaf' article with management guidelines for root rot diseases of lettuce.

- A short DVD demonstrating disease suppression in hydroponic lettuce by a microbial biocontrol containing the bacterium, *Bacillus subtilis*.

- A 'Vegetables Australia' article summarising the major project achievements.

Recommendations

Sustained and effective management of root rot diseases of hydroponic lettuce will require an ongoing commitment by growers to minimise the predisposing factors identified in this study. In particular, it is recommended that growers adopt sound hygiene and sanitation practices to prevent pathogens establishing in their units and to reduce the risk of them spreading. Using a few lettuce of the very susceptible cultivars identified in this project could be a useful way of monitoring for early disease expression in production units. Use of larger seedling plug sizes were shown to result in larger plants in the presence of plant pathogens, however they need further evaluation under conditions of high disease pressure. The use of microbial biocontrols should be encouraged but further studies are required to determine optimum use-patterns. Of the other potential biocontrols assessed in this project, *Pseudomonas putida* and *Streptomyces lycius* were shown to give intermediate control of root rots. These and other potential biocontrols should be tested further and their compatibility assessed as mixed formulations. Some overseas studies have demonstrated compatible combinations of microbial biocontrols that act in a synergistic way, thereby increasing their efficacy.