FINAL REPORT

Effective management of root diseases in hydroponic lettuce

HAL Project VG04012

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

April 2008

AUSVEG

NSW DEPARTMENT OF PRIMARY INDUSTRIES

Vegetable R&D
Your national vegetable levy at work

HAL
Know-how for Horticulture™

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

HAL

Know-how for Horticulture™

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 4
Media Summary 5
Media Summary 5
Technical Summary 6
Introduction 8
Aims: 9
~~Materials & Methods ..... 10~~
~~Materials & Methods ..... 10~~
~~1. Farm surveys & laboratory diagnosis ..... 10~~
~~1.1 Farm surveys ..... 10~~
~~1.2 Laboratory diagnosis ..... 10~~
~~1.2.1 DNA extraction from isolates ..... 10~~
~~1.2.2 Genetic analysis of isolates ..... 10~~
~~2. Experiments to determine pathogenicity & product efficacy ..... 11~~
~~Table 1. Trials established for VG04012 ..... 11~~
~~2.1 Pathogenicity Trials ..... 14~~
~~2.2 Disinfection Efficacy trials ..... 14~~
~~2.2.1 Iodine ..... 14~~
~~2.2.2 Calcium hypochlorite and Chlorine dioxide (ClO_2) ..... 15~~
~~2.2.3 The quaternary ammonium disinfectant, Sporekill™ (didecylidimethyl ammonium chloride) ..... 15~~
~~2.2.4 The non-ionic wetting agent, Agral® ..... 15~~
~~2.2.5 Monochloramine, PythOff® ..... 16~~
~~2.2.6 Sonication & Ultra-violet (UV) disinfection ..... 16~~
~~2.3 Microbial biocontrol & growth stimulant efficacy trials ..... 17~~
~~Table 2. Microbial biocontrols & growth stimulants tested ..... 17~~
~~2.3.1 Trial #3: Microbial biocontrol products and *Phytophthora drechsleri* on four hydroponic lettuce cultivars ..... 17~~
~~2.3.2 Trial #5: Microbial inoculant *FZ Plus* and *Pythium spp.* ..... 18~~
~~2.3.3 Trial# 12: Microbial inoculants *FZ Plus* verses *Phytophthora* on 1 lettuce cultivar (Brown mignonette) with and without heating of nutrient solution. ..... 18~~
~~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. ..... 19~~
Results & Discussion 20
1. Farm surveys & laboratory diagnosis 20
~~2. Pathogenicity Trials ..... 21~~
~~Table 3. Mean lettuce wet weights from pathogen treatments ..... 22~~
~~Table 4. Mean lettuce wet weights from pathogen treatments ..... 22~~
~~2.2 Disinfection Efficacy Trials ..... 22~~
~~2.2.1 Iodine ..... 22~~
~~2.2.2 Calcium Hypochlorite and Chlorine dioxide (ClO_2) ..... 23~~
~~2.2.3 The quaternary ammonium disinfectant, Sporekill™ ..... 23~~
~~Table 5. Lettuce wet weights for Trial #25 ..... 23~~
~~2.2.4 The non-ionic wetting agent, Agral® ..... 24~~
~~2.2.5 Monochloramine, PythOff® ..... 24~~
~~2.2.6 Sonication & Ultra-violet (UV) disinfection ..... 24~~

Table 6. Recovery of plant pathogens from agar baits or colony-forming units (c.f.u.) on filter paper incubated on PPA medium 24
Table 7. Recovery of *Fusarium* and *Pythium* from water samples taken on the day of inoculation (8/11/06) 25
Table 8. Recovery of *Fusarium* and *Pythium* from water samples (14/11/06) 25
Table 9. Recovery of *Fusarium* and from water samples (22/11/06) 26
4. Microbial biocontrol & growth stimulant efficacy trials 26
Table 10. Effect of microbial inoculants and *Phytophthora drechsleri* on wet weights of four lettuce cultivars 27
Table 11. Effect of biological and chemical treatments and *Pythium* on wet weights of two lettuce cultivars for Trial #5 27
Table 12. Lettuce (cv. *Brown Mignonette*) wet weights for Trial #12 ... 28
Table 13. Mean wet weights of lettuce for Trial #15 28
Table 14. Mean lettuce fresh weights for Trial #16 29
Table 15. Mean lettuce fresh weights for Trial #18 29
References 31
Technology Transfer 33
Recommendations 34

List of tables and figures

Table 1. Trials established for VG04012 11
Table 2. Microbial biocontrols & growth stimulants tested 17
Table 3. Mean lettuce wet weights from pathogen treatments 22
Table 4. Mean lettuce wet weights from pathogen treatments 22
Table 5. Lettuce wet weights for Trial #25 23
Table 6. Recovery of plant pathogens from agar baits or colony-forming units (c.f.u.) on filter paper incubated on PPA medium 24
Table 7. Recovery of *Fusarium* and *Pythium* from water samples taken on the day of inoculation (8/11/06) 25
Table 8. Recovery of *Fusarium* and *Pythium* from water samples (14/11/06) 25
Table 9. Recovery of *Fusarium* and from water samples (22/11/06) 26
Table 10. Effect of microbial inoculants and *Phytophthora drechsleri* on wet weights of four lettuce cultivars 27
Table 11. Effect of biological and chemical treatments and *Pythium* on wet weights of two lettuce cultivars for Trial #5 27
Table 12. Lettuce (cv. *Brown Mignonette*) wet weights for Trial #12 28
Table 13. Mean wet weights of lettuce for Trial #15 28
Table 14. Mean lettuce fresh weights for Trial #16 29
Table 15. Mean lettuce fresh weights for Trial #18 29

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.

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.