Project 2.2 Best-practice IPM Strategies for Control of Major Soilborne Diseases of Vegetable Crops throughout Australia

Dr Ian Porter
Victorian Department of Primary Industries (VICDPI)

Project Number: VG07125

VG07125

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 vegetables industry.

All expressions of opinion are not to be regarded as expressing the opinion of Horticulture Australia Ltd or any authority of the Australian Government.

The Company and the Australian Government accept no responsibility for any of the opinions or the accuracy of the information contained in this report and readers should rely upon their own enquiries in making decisions concerning their own interests.

ISBN 0 7341 2576 3

Published and distributed by:
Horticulture Australia Ltd
Level 7
179 Elizabeth Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399

HAL

Horticulture Australia

Best-practice IPM strategies for control of major soilborne diseases of vegetable crops

Final report for project VG 07125

Caroline Donald *et al.*

(Dec 2010)

Victoria
The Place To Be

DEPARTMENT OF PRIMARY INDUSTRIES | biosciences research

Project Details

Best Practice IPM strategies for control of major soilborne diseases of vegetable crops.

Final report for Horticulture Australia Ltd project VG07125
Dec 2010

This report details research directed towards the development of IPM based strategies for the management of key soilborne diseases (caused by the pathogens *Sclerotinia, Pythium, Fusarium* and *Rhizoctonia* spp.) in vegetable crops. It includes research into a range of techniques which may be applied broadly across a number of pathogens (eg. biofumigation and green manures) and others which are specific to a particular host or pathogen (eg. chemical controls and grafting). A significant part of the work has been directed towards the development and preliminary evaluation of 'novel' strategies such as plant and fungal derived volatiles, melanin inhibitors, plant defence activators and the use of nutrients and surfactants. There have been many contributors to this body of work, their contributions to each part of the work are acknowledged at the beginning of each chapter.

Project Leaders: Caroline Donald and Ian Porter
Department of Primary Industries, Victoria
Biosciences Research Division
Private Bag 15
Ferntree Gully Delivery Centre, VIC, 3156
Phone: (03) 9210 9222 Fax: (03) 9800 3521 Email: caroline.donald@dpi.vic.gov.au

Contributing research agencies and project staff:
Department of Primary Industries, Victoria
Dr Caroline Donald, Dr Oscar Villalta, Dr Ian Porter, Dr Scott Mattner, Mr Ross Mann, Ms Cassie Scoble, Ms Denise Wite, Mr David Riches

Queensland Department of Employment, Economic Development and Innovation
Dr Cherie Gambley, Dr Christine Horlock

Northern Territory Department of Resources
Mr Barry Condè, Mr Mark Traynor, Dr Lucy Tran-Nguyen, Mr Sean Bithell

New South Wales Department of Industry and Investment
Mr Len Tesoriero, Dr Leanne Forsyth

La Trobe University
Dr Kim Plummer, Ms Alicia Greenhill

Funding for this project was provided by Horticulture Australia Limited (HAL) using vegetable industry levy and federal government sources, the Department of Primary Industries Victoria, Queensland Dpartment of Primary Industries and Fisheries, Northern Territory Department of Primary Industries, Fisheries and Mines and the New South Wales Department of Primary Industries.

← Image previous page, report cover – incorporation of biofumigant and green manure crops at the Clyde VIC field trial site.

Acknowledgements

There were many people and organisations that provided assistance to make this research possible. The project team gratefully acknowledges the assistance provided by the following:

- The vegetable growers that have worked with us graciously allowing field trials on their farms and providing assistance in their establishment, maintenance and harvest. Murray Ingram, Tony and Rocky Lamattina, Greg and Rick Butler, Deborah and Darren Corrigan (Victoria), Van Son Chau, An Tran and Van Nguyen (Northern Territory), Joe Boustani (New South Wales).
- Craig Arnott and staff for modifying their equipment for demonstration purposes and lending it, together with an operator to pulverise biofumigant and green manure crops on two occasions for a trial at Clyde.
- Rod Jones and Bret Henderson (Victorian Department of Primary Industries) for assistance with freeze drying and glucosinolate analysis from plant tissues.
- Gavin Rose and David Allen (Victorian Department of Primary Industries, Food & Health Sciences-Agrifood Production Systems, Future Farming Systems Research Division) for conducting isothiocyanate analysis from soil samples collected from IPM trials.
- Fiona Thomson (Victorian Department of Primary Industries), Mark Hearnden (NT Department of Resources) for biometric services.
- Dale Cumberland and Lois Ulyatt (Plant Pathology Branch, Dept of Resources NT), Jon Collins, Tehgan Crowe and Kulan Turton, (Industry and Investment NSW), Jorge Ghiano, Vijay Chandoula, Sahil Balotra (Department of Primary Industries Victoria), Lynette Haselgrove, Dalphy Harteveld, Emma Ballard, Joy Conroy (Queensland Department of Employment, Economic Development and Innovation) for technical assistance.
- Jacqueline Edwards for her critical review of this report and other written material produced during the life of the project and her assistance at the Lindenow field day.

Published by the Department of Primary Industries
Bioprotection, March 2010

Authorised by the Department of Primary Industries
1 Spring Street, Melbourne 3000.

Contents

MEDIA SUMMARY **8**

TECHNICAL SUMMARY **9**

RECOMMENDATIONS **10**

1. LITERATURE REVIEW AND PATHOGEN WORKSHOPS **13**

1.1 INTRODUCTION 13
1.2 CURRENT FUNGICIDES AND THEIR MODES OF ACTION 17
1.2.1 Multi site (Protectant) fungicides *17*
1.2.2 Inhibitors of nucleic acid synthesis *18*
1.2.3 Inhibitors of mitosis and cell division *18*
1.2.4 Inhibitors of respiration *18*
1.2.5 Inhibitors of amino acid and protein synthesis *19*
1.2.6 Disruption of signal transduction *19*
1.2.7 Inhibitors of lipids and membrane synthesis *19*
1.2.8 Inhibition of sterol synthesis *19*
1.2.9 Unknown mode of action *19*
1.3 CHEMICAL CONTROL OF SOILBORNE PLANT PATHOGENS IN AUSTRALIA 20
1.4 CULTURAL PRACTICES 21
1.4.1 Crop hygiene *21*
1.4.2 Crop rotation and planting sequence *21*
1.4.3 Planting date and crop density *22*
1.4.4 Tillage and residue management *22*
1.4.5 Rouging *22*
1.4.6 Heat and solarisation *23*
1.5 BIOLOGICAL SOIL DISINFESTATION 23
1.6 SOIL PH 24
1.7 MINERAL NUTRITION 24
1.7.1 Nitrogen, phosphorous and potassium (NPK) *24*
1.7.2 Other important macronutrients *26*
1.7.3 Micronutrients *27*
1.8 ORGANIC AMENDMENT 28
1.8.1 Composts *28*
1.8.2 High nitrogen soil amendments *29*
1.8.3 Green manures and 'biofumigants' *30*
1.8.4 Other organic amendments *31*
1.9 DISEASE RESISTANCE/TOLERANCE 31
1.9.1 Genetic resistance *31*
1.9.2 Grafting *32*
1.10 SURFACTANTS AND BIOSURFACTANTS 32
1.11 PLANT DERIVED COMPOUNDS 33
1.12 BIOLOGICAL CONTROL 35
1.12.1 Suppressive soils *35*
1.12.2 Plant Growth Promoting Rhizobacteria (PGPR), biological control agents (BCA) and endophytes *36*
1.12.3 Mycorrhizal fungi *42*
1.13 SELF DEFENCE 42
1.14 INDUCED RESISTANCE 44
1.14.1 Systemic acquired resistance (SAR) *45*
1.14.2 Natural elicitors *45*
1.14.3 Synthetic elicitors *47*
1.14.4 Induced systemic resistance *47*
1.15 GENETIC ENGINEERING 48
1.16 PROGRESS TOWARD INTEGRATED CONTROL OF SOILBORNE PLANT PATHOGENS 48
1.17 ACKNOWLEDGEMENTS 51
1.18 REFERENCES 52
1.19 APPENDIX 1.1 – IPM BASED RESEARCH PRIORITIES IDENTIFIED AT PATHOGEN SPECIFIC WORKSHOPS 74

1.19.1 Sclerotinia *74*
1.19.2 Rhizoctonia (vegetable and potatoes) *76*
1.19.3 Fusarium and Pythium *77*
1.20 APPENDIX 1.2 – 'BEST PRACTICE' IPM STRATEGIES FOR KEY SOILBORNE PATHOGENS IN AUSTRALIA. RESEARCH PIRORITIES FITTED AGAINST PROJECT MODULES 78
1.20.1 Sclerotinia *78*
1.20.2 Pythium *80*
1.20.3 Fusarium *81*
1.20.4 Rhizoctonia *82*

2. IPM STRATEGIES FOR FIELD GROWN VEGETABLE CROPS 84

2.1 INTRODUCTION 85
2.2 MATERIALS AND METHODS 85
2.2.1 Plant treatments *85*
2.2.2 Glucosinolate (GSL) concentrations *86*
2.2.3 Pathogen suppression in vitro *87*
2.2.4 Host susceptibility studies *87*
2.2.5 The effect of brassica treatments on pathogen control in pots *87*
2.2.6 Field trials - influence of crop rotation and biofumigation on inoculum, disease, yield and soil condition *88*
2.3 RESULTS AND DISCUSSION 90
2.3.1 Concentrations of glucosinolate (GSL) in brassica tissue *90*
2.3.2 The effect of brassica treatments on pathogen mycelial growth in culture *93*
2.3.3 The effect of biofumigant treatments on disease control in pots *96*
2.3.4 Field trials - the effect of rotation and biofumigation on inoculum, disease and yield *97*
2.3.5 Host susceptibility *110*
2.4 CONCLUSIONS 113
2.5 ACKNOWLEDGEMENTS 115
2.6 REFERENCES 115

3. IPM STRATEGIES FOR SOILLESS VEGETABLE PRODUCTION SYSTEMS 118

3.1 INTRODUCTION 118
3.2 RAPID SCREENING OF NOVEL AND DEVELOPING TREATMENTS – SPINACH AND CORIANDER (NSW) 120
3.2.1 Materials and Methods *120*
3.2.2 Results & Discussion *121*
3.2.3 Conclusions *124*
3.3 RAPID SCREENING OF NOVEL AND DEVELOPING TREATMENTS – CUCUMBER (VIC) 124
3.3.1 General Materials and Methods *124*
3.3.2 Systemic acquired resistance and biological control in cucumber *125*
3.3.3 Surfactants for managing Pythium diseases in cucumber *127*
3.3.4 Conclusions *129*
3.4 ON-FARM EVALUATION OF CHEMICAL DRENCHES TO MANAGE *FUSARIUM* AND *PYTHIUM* DISEASES IN CUCUMBER (NSW) 130
3.4.1 Materials and methods *130*
3.4.2 Results and Discussion *131*
3.5 ACKNOWLEDGEMENTS 132
3.6 REFERENCES 133

4. GRAFTING AND *FUSARIUM* WILT OF SNAKE BEAN 136

4.1 INTRODUCTION 136
4.2 MATERIALS AND METHODS 138
4.2.1 Grafting workshop and farm visits *138*
4.2.2 On-farm field trials *138*
4.2.3 Isolate collection and characterisation *139*
4.2.4 Isolate characterisation/race determination *139*
4.2.5 Molecular characterisation *140*
DNA extractions 140
Intergenic spacer (IGS) region PCR 141
Restriction fragment length polymorphism 142
Random Amplified Polymorphic DNA 142
Multigene characterisation - EF1-α, ß tubulin and histone 3 PCR 143
Multigene characterisation - Sequencing 143
4.3 RESULTS 143

4.3.1 Grafting workshop and farm visits *143*
4.3.2 On-farm field trials and demonstrations *144*
4.3.3 Isolate cultures *147*
4.3.4 Race determination *147*
4.3.5 Molecular characterisation *153*
4.4 DISCUSSION 158
4.4.1 Effectiveness and uptake of grafting to manage Fusarium wilt of snakebeans in the Northern Territory *158*
4.4.2 Isolate characterisation/race determination *158*
4.4.3 Molecular characterisation *159*
4.5 GRAFTING REVIEW – THE POTENTIAL OF GRAFTING FOR SOILBORNE DISEASE MANAGEMENT IN AUSTRALIAN VEGETABLE PRODUCTION SYSTEMS 160
4.5.1 Summary *160*
4.5.2 Introduction *161*
4.5.3 Use of grafting *161*
Extent of soilborne pathogen management provided by grafting 162
Importance of rootstock:scion compatibility 163
Grafting and after-care methods 164
New technologies 165
Management of diseases other than soilborne pathogens 166
Non-disease management benefits from grafting 166
Soilborne pathogens in Australian crops managed with grafting 166
4.5.4 Case study 1: Tomato grafting in the Northern Territory *167*
4.5.5 Case study 2: Snakebean grafting in the Northern Territory *168*
4.5.6 Factors affecting uptake of grafting *169*
4.5.7 Potential for grafting in other Australian vegetable crops *169*
Management potential for fungal soilborne pathogens in Australian crops by grafting 169
4.5.8 Determining feasibility *172*
Cost:benefit balance 172
Basis for cost comparisons of grafting 173
Potential limitations 174
4.5.9 Conclusions *175*
4.6 ACKNOWLEDGEMENTS 175
4.7 REFERENCES 176
APPENDIX 4.1 OVERSEAS REPORTS OF GRAFTING FOR SOILBORNE PATHOGEN MANAGEMENT IN VEGETABLE PRODUCTION 185
APPENDIX 4.2 ESTIMATED COSTS OF SNAKEBEAN PRODUCTION, YIELD LOSSES TO SNAKEBEAN WILT, GRAFTING COSTS AND NET RETURNS FROM GRAFTED PLANTS 188

5. PLANT DERIVED COMPOUNDS 191

5.1 INTRODUCTION 191
5.2 *IN-VITRO* (LABORATORY) SCREENING TO EVALUATE ANTIMICROBIAL ACTIVITY 192
5.2.1 Materials and methods *192*
5.2.2 Results *196*
5.3.2 Discussion *204*
5.3 POT BIOASSAYS TO DETERMINE THE DISEASE CONTROL POTENTIAL OF SELECTED PLANT-DERIVED COMPOUNDS 206
5.3.1 Materials and Methods *206*
5.3.2 Results and Discussion *207*
5.4 FIELD STUDIES TO DETERMINE THE DISEASE CONTROL POTENTIAL OF SELECTED PLANT-DERIVED COMPOUNDS 211
5.4.1 Materials and Methods *211*
5.4.2 Results *213*
5.4.3 Discussion *215*
5.5 CONCLUSIONS 216
5.6 REFERENCES 216

6. DISRUPTION OF FUNGAL RESTING STRUCTURES 219

6.1 INTRODUCTION 219
6.2 THE ROLE OF MELANIN IN SCLEROTIAL FORMATION 222
6.2.1 Chemical inhibition of melanin *222*
6.2.2 Expression of melanin biosynthesis genes *227*
6.2.3 Knock out of melanin biosynthesis genes *228*
6.2.4 Conclusions *229*
6.3 SCLEROTIA ABERRANT MUTANTS 230
6.3.1 Phenotyping sclerotia-aberrant mutants *230*
6.3.2 Identification of T-DNA insertions points *231*

6.3.3 Conclusions *232*
6.4 REFERENCES 232

7. FUNGAL DERIVED VOLATILES (ENDOPHYTES FROM NATIVE SPECIES) 235

7.1 INTRODUCTION 235
7.2 MATERIALS AND METHOD 236
7.2.1 Endophytes *236*
7.2.2 Genetic Diversity of Endophytes *236*
7.2.3 Bioactivity *237*
7.2.4 Analysis of Endophyte Volatile Metabolites *237*
7.2.5 Mycofumigation Pot Trial *237*
7.3 RESULTS AND DISCUSSION 238
7.3.1 Genetic Diversity of Endophytes *238*
7.3.2 Bioactivity *238*
7.3.3 Analysis of Endophyte Volatile Metabolites *239*
7.3.4 Mycofumigation Pot Trial *243*
7.4 CONCLUSIONS 243

8. PRELIMINARY EVALUATION OF OTHER NOVEL COMPOUNDS 245

8.1 INTRODUCTION 245
8.2 MATERIALS AND METHODS 247
8.2.1 Products evaluated *247*
8.2.2 In vitro assays *248*
8.2.3 Pot trial general methods *248*
8.2.4 Pot trials for biofumigant evaluation *248*
8.2.5 Pot trials for evaluation of nutrient, SAR and surfactant products *249*
8.2.6 Seed treatments with nutrient and SAR products *249*
8.2.7 Statistical analyses *249*
8.3 RESULTS 250
8.3.1 In vitro assays *250*
8.3.2 Pot trials for evaluation of plant derived products *251*
8.3.3 Pot trials to evaluate nutrient and SAR products *252*
8.3.4 Pot trials to evaluate surfactant products *256*
8.3.5 Phytotoxic effects of test products *258*
8.4 DISCUSSION 260
8.5 REFERENCES 261

9. TECHNOLOGY TRANSFER 263

9.1 KEY COMMUNICATION ACTIVITIES 263
9.2 EVALUATION OF COMMUNICATION ACTIVITIES 269
9.3 TECHNOLOGY TRANSFER RECOMMENDATIONS 271

Media Summary

This project has provided dramatic reductions (of up to 98 %) in the impact of soilborne diseases in vegetable cropping systems using non-chemical control strategies such as crop rotation and grafting. It has demonstrated that:

- the in-field effects of crop rotation using *Brassica* biofumigant crops include excellent weed suppression, a reduction of root rots in green beans (by up to 35 %) and an increase in the fresh weight of spring onions (by up to 16 %).
- grafting snake bean onto the *Fusarium* resistant Iron cowpea rootstock can reduce the incidence of *Fusarium* wilt in this crop by 98 %.
- four recently available *Brassica* biofumigant crops (Caliente mustard 199, Mustclean, Gladiator and Nemfix) with higher levels of isothiocyanate (ITC) producing glucosinolate (GSL) compounds than common varieties were the most effective against four major soilborne pathogens of vegetables (*Sclerotinia, Rhizoctonia, Pythium* and *Fusarium* spp.).
- crop rotation strategies can reduce inoculum of soilborne pathogens by breaking the disease cycle, biofumigation activity (eg. mustards) and/or improving soil health.
- biofumigant crops should be pulverised before incorporation into moist soil to ensure biofumigant compounds are released into the soil.
- some green manure crops produce other beneficial effects including increased soil organic matter, nitrogen and biological activity.

A four page guide 'Managing soilborne diseases in vegetables' has been produced and distributed as a package of Vegetable IPM Disease Program notes to over 300 growers at the six field days and seven national workshops conducted during the last two years of the project.

The economic feasibility of crop rotation with *Brassica* green manure crops was shown to be optimised by avoiding winter and summer plantings which may suffer from slow growth and heavy pest pressure respectively. Also, since *Brassica juncea* green manures are highly susceptible to *Plasmodiophora brassicae,* sites with a history of clubroot disease should be avoided. Grafting was shown to be economically feasible where infection levels are moderate to severe as yields from grafted plants can be twice as high as non-grafted seedlings. Under low disease pressure, the yield increases are smaller and less likely to offset the extra costs (mainly labour) of producing grafted plants.

A second component of the project has been the evaluation of novel control strategies as future IPM tools. Much of this work was conducted by post-graduate students associated with the project. The range of treatments evaluated included biological controls, novel chemical controls, nutrients, surfactants, plant and fungal (endophyte) derived volatile compounds and inducers of systemic acquired resistance. From these, plant derived volatile compounds, particularly those based on thyme, clove bud, origanum and mustard (eg. Vigor® and ECO-V) oils and mycofumigation with endophytes that produce volatile antimicrobial compounds are considered worthy of further development as future IPM tools for field application. Liquid chitin, chitosan, and *Bacillus subtilis* (biological control) in conjunction with phosphorous acid are worthy of further development for hydroponic applications.

Technical Summary

Since the Australian vegetable industry produces a large number of crops which are affected by a diverse range of pathogens the research has taken a 'systems' approach to the management of soilborne diseases seeking to develop strategies and techniques which may be applied broadly in vegetable production systems against soilborne diseases in general, rather than specifically against one pathogen in a single host crop. A 2007 pathology gap analysis conducted for the Australian vegetable industry (VG06092) identified *Sclerotinia, Rhizoctonia, Pythium* and *Fusarium* spp. as the four most economically important soilborne pathogens nationally. The development of new IPM tools and control strategies to manage these pathogens in vegetable production systems is reported.

An important component of the project has been the development of practical crop rotation strategies that reduce the impact of soilborne diseases. This work was conducted in three stages, *in-vitro* screening, pot bioassays and field trials. Four new *Brassica* green manure crops (Caliente 199, Mustclean, Gladiator and Nemfix) were identified with high concentrations of volatile glucosinolates. These treatments completely killed pathogen mycelium in vitro, were as good as or superior to the standard Fumafert® and BQ Mulch™ treatments and were more effective than another seven Brassicas evaluated.

Pot trials confirmed the biofumigant potential of Nemfix, Gladiator, Caliente 199 and Mustclean for controlling *S.minor* infection and Nemfix and Mustclean for controlling *R. solani.* In the field *Brassica* green manures reduced the severity of root rot in green beans by up to 35% compared with fallow or grass and cereal rotations, provided excellent weed suppression and improved a range of soil health parameters. The legume crop Faba bean was also a good rotation choice providing similar levels of disease control but having less effect on weeds. Preliminary results indicate that soil biofumigation with *Brassica* green manure crops and crop rotation could be useful tools to manage soilborne pathogens and increase productivity. For optimum benefit growers should integrate rotation with other IPM tools, avoid winter (as the crops grow too slowly) and summer (due to insect pest pressure) plantings and sites infested with the clubroot pathogen (as *Brassica juncea* green manures are highly susceptible to this pathogen).

In the Northern Territory field trials using snake beans grafted onto a *Fusarium* resistant Iron cowpea rootstock have reduced the incidence of *Fusarium* wilt by as much as 98 %. It is likely to be economically advantageous to use these grafted plants rather than non-grafted seedlings where infection levels are moderate to severe. Under such conditions grafted plants are likely to yield twice as much as non-grafted seedlings. Under low disease pressure, the yield increases are less likely to offset the extra costs (mainly labour) of producing grafted plants. There is potential for grafting to be applied more widely to 'high value' seedlings (eg. cucurbits, capsicums). Screening studies to identify candidate host crops and sources of resistant, compatible rootstocks are recommended.

A second component of the project has been the identification and evaluation of novel control treatments and strategies with potential for further development as future IPM tools. Products were evaluated by *in-vitro* screening and pot or hydroponic trials. The range of treatments evaluated included biological controls, chemical controls, nutrients, surfactants, plant derived volatile compounds, fungal (endophyte) derived volatile compounds and inducers of systemic acquired resistance. From these the following are considered worthy of further development as future IPM tools:

- Plant derived volatile compounds particularly those based on thyme, clove bud, origanum and mustard (eg. Vigor® and ECO-V) oils. In particular methods which retain the product within treated soils to optimise efficacy and reduce worker exposure to volatiles need to be developed.
- Endophytes that produce volatile antimicrobial compounds for mycofumigation.
- Liquid chitin and chitosan which consistently reduced the severity of disease caused by *P. irregulare* in hydroponic cucumbers. Trials could be extended to a wider range of crops and pathogens.
- *Bacillus subtilis* (biological control)/phosphorous acid for hydroponic applications. Trials could be extended to a wider range of crops and pathogens.

This project was a foundation project of the vegetable IPM disease program. The program approach has brought together researchers from all over Australia and improved the efficiency of use of levy funds. It has created opportunity for interaction, critical discussions and planning. It has also provided a focus for and coordinated approach to technology transfer/communication activities. The program approach should be strongly encouraged as we enter Phase II.

Recommendations

Based on the research in this project and ensuing compatability with other of the National IPM projects, the following is recommended:

- Vegetable growers consider trialling and assessing the economic impact of the new *Brassica* green manure crops for their biofumigant potential to reduce soilborne pathogens of vegetables. Consideration of their flowering period is important - for instance during cool periods of the year, Mustclean was shown to flower 60 days after sowing and Caliente 199 and BQ Mulch™ 90 days after sowing.

- *Brassica* green manure crops are most effective when treated and incorporated properly, which includes being pulverised before incorporation to optimise the release of chemical compounds from the plant tissues. A rotary hoe will be inadequate to do this effectively; specialised equipment ie. a flail type mulcher or other specialised machinery is required.

- The benefits of using biofumigant crops on soil health also be assessed. A grower in eastern Victoria who has grown Mustclean and Gladiator reported improvements in soil structure and water infiltration. For best results avoid either winter plantings as the crops grow too slowly or summer plantings which are subject to heavier insect pest pressure. Also avoid sites infested with the clubroot pathogen (as *Brassica juncea* green manures are highly susceptible to this pathogen).

- Snake bean growers who have in the past experienced moderate to severe *Fusarium* wilt disease pressure consider grafting snake bean seedlings onto the resistant Iron cowpea root stock. This root stock is also resistant to root knot nematode.

- Industry may wish to consider researching or expanding the use of grafting for other candidate crops (eg. cucurbits, capsicums) which are moderately to severely affected with serious soilborne disease problems. The study should also consist of a review of the sources of resistance and the compatibility of the root stocks.

- The project has identified several promising areas which require further development as future IPM tools in a second phase of this project. Specifically,

    - Methods are required which retain volatile plant derived products within treated soils to optimise efficacy and reduce worker exposure to volatiles.

    - Commercially suitable methods of preparing endophyte products for field application, together with in-field efficacy and survival studies are required to improve their usefulness for field application and use.

    - Large scale controlled hydroponic studies are required to evaluate liquid chitin, chitosan and *Bacillus subtilis* in conjunction with phosphorous acid against a wider range of host crops and pathogens than could be covered in this study.

- It is strongly suggested that research on novel methods to reduce soilborne inoculum of the major pathogens be continued as, in the absence of soil fumigation, there are no effective measures to control soilborne pathogens and new IPM mechanisms are required to reduce the $50M loss estimated to be caused by these major soilborne pathogens throughout the national vegetable industry.

- It is critical that phase II of this project continue in order to achieve maximum benefit for the industry as many findings presented in this report (eg. the use of green manures including *Brassica* biofumigants) are the result of one crop rotation cycle. Many of the anticipated effects on soilborne diseases and soil health parameters are likely to be cumulative and more positive (or negative) effects may result from repeated use of the IPM strategies reported above. It is strongly recommend that the long term trial sites be retained and the work on them supported so that important long term effects can be determined.

This project was a foundation project of the vegetable IPM disease program. The program approach has brought together researchers from all over Australia. It has created opportunity for interaction, critical discussions and planning. It has also provided a focus for and coordinated approach to technology transfer/communication activities. The program approach should be strongly encouraged as we enter Phase II.

Literature review and pathogen workshops

1. Literature review and pathogen workshops

Towards sustainable integrated control of soilborne diseases in vegetable crops
A review of available and emerging chemical and non-chemical strategies and their potential compatibility with IPM programs

By Caroline Donald[1], Christine Horlock[2], Barry Condè[3], Oscar Villalta[1] and Ian Porter[1]
[1] Department of Primary Industries, Victoria
[2] Queensland Department of Employment, Economic Development and Innovation
[3] Northern Territory Department of Resources

At the beginning of the project a review of existing and emerging chemical and non-chemical treatments for the control of key soilborne diseases of vegetable crops in Australia and overseas was undertaken. Three pathogen specific workshops attended by researchers, consultants, representatives of the chemical industry and key growers were held during 2007 - Rhizoctonia (16-17 Oct, Victoria), Pythium & Fusarium (23 Nov, New South Wales) and Sclerotina (28-29 Nov, Tasmania). Information from these workshops (Tables 1.1 & 1.2 and 1.19 Appendix 1.1) was pooled with that from traditional literature sources to create a review of available and emerging chemical and non-chemical strategies and their potential compatibility with IPM programs. This information was used to set research priorities and complete a program of research for HAL pathology subprogram 2.2 directed towards 'Best practice management strategies for the control of soilborne diseases in vegetable crops' (1.20 Appendix 1.2).

1.1 Introduction

The intensification of vegetable production in Australia and elsewhere has increased the impact of soilborne diseases. Seedling damping off, rots and wilts are problems common to a range of vegetable crops in most of Australia's major vegetable production regions. A recent pathology gap analysis conducted by the Australian vegetable industry identified *Sclerotinia, Pythium, Fusarium* and *Rhizoctonia* as the industry's most significant soilborne pathogens. These pathogens cause significant diseases in a range of vegetable crops (Table 1.1). In late 2007, a series of workshops attended by experts on specific pathogens and industry representatives (chemical industry representatives, crop consultants, vegetable processors and key growers) explored the impact of these diseases and prospects for their control (see Acknowledgements for participant list). These groups identified key disease issues caused by *Sclerotinia, Pythium, Fusarium* and *Rhizoctonia* in Australia (Table 1.1) and current control strategies (Table 1.2). It was clear from these meetings that current control strategies, where they exist, are based predominantly on the use of chemical treatments such as fumigants and fungicides (Table 1.2).

This dependence of the vegetable industry on chemicals to manage soilborne diseases leaves it vulnerable to regulatory change, chemical resistance and the economic priorities of chemical companies. For example, due to the large number of relatively low value vegetable crops affected by *Sclerotinia, Pythium, Fusarium* and *Rhizoctonia* often effective chemicals are not registered for the target crop and effective control depends upon minor use permits. Often if registrations are available for the target host crop and pathogen, these registrations exclude greenhouse use (eg. *Pythium* root rots

← Image previous page, chapter 1 cover sheet – project leaders Caroline Donald and Ian Porter conduct a preliminary project development meeting to identify priority pathogens and R & D gaps.

and wilts of greenhouse cucumbers), the fungicides are rendered ineffective due to chemical resistance in the pathogen (eg. Fusarium wilt of snow pea) or the products are withdrawn (eg. benomyl for management of Fusarium wilt of cucumbers and procymidone for control of *S. minor* in lettuce). In addition to the problems posed by a lack of available, effective, registered chemicals, increasingly chemical use in the vegetable industry is being scrutinised and growers face market pressure to minimise chemical use and adopt more sustainable, less exploitive agricultural systems.

A range of Integrated Pest Management (IPM) based research priorities for the control of *Sclerotinia, Pythium, Fusarium* and *Rhizoctonia* diseases were identified at the pathogen specific workshops (Appendix 1.1). This review of the available literature concerning existing and emerging chemical and non-chemical treatments in Australia and overseas was conducted to compliment the information gained from local experts at the various workshops. The information gained from both sources has been used to set research priorities and compile a program of research for HAL pathology subprogram 2.2 directed towards the development of 'Best practice management strategies for the control of soilborne diseases in vegetable crops' (Appendix 1.2).

Table 1.1. Key soilborne pathogens and the diseases they cause in Australian vegetable production systems.

Pathogen	Crop	Disease
Sclerotinia		
Sclerotinia minor	Lettuce	Lettuce drop
Sclerotinia sclerotiorum	Beans	White mould
	Brassicas	White mould or soft rot
	Potatoes	White mould or Sclerotinia rot
	Lettuce	Lettuce drop
	Cucumber	Stem and fruit rot
	Tomato	Stem and fruit rot
	Artichoke	Basal stem rot
	Carrots	Sclerotinia rot, crown rot
Pythium		
P. ultimum P. sylvaticum. P. dissotocum or *P. violae*	Lettuce seedlings	Damping off
P. ultimum, P. aphanidermatum, P. irregulare, *P. myriotylum, P. spinosum, P. mamillatum*	Cucumber seedlings	Damping off
P. myriotylum or *P. aphanidermatum*	Capsicum	Sudden wilt
P. sulcatum or *P. violae*	Carrots	Cavity spot
P. dissotocum, P. coloratum	Lettuce	Root rot
P. ultimum, P. aphanidermatum, *P. myriotylum, P. spinosum*	Cucumber	Root rot & sudden wilt
P. dissotocum (and others)	Beans	Root rot
Fusarium		
Fusarium spp.	Vegetable seedlings	Damping off
F. oxysporum f.sp. *pisi* (races 1 & 6)	Snow Pea	Fusarium wilt (true wilt & near wilt)
		Fusarium wilt & crown rot
F. oxysporum f. sp. *basilica*	Sweet and Thai basil	Fusarium wilt
F. oxysporum f. sp. *cucumerinum* (Foc), or possibly (and less frequently) *F. oxysporum* f. sp. *radicis-cucumerinum*	Cucumber	
F. oxysporum f.sp. *tracheiphilum*	Snake bean	Fusarium wilt
F. oxysporum f. sp. *lycopersici*	Tomatoes	Fusarium wilt & root rot
F. oxysporum, *Fusarium avenaceum*	Leeks	Fusarium foot rot
F. verticillioides, F. proliferatum, F. subglutinans	Sweet corn	Fusarium cob rot
Fusarium spp.	Beans	Fusarium wilt & root rot
	Carrots	Crown rot
	Parsley	
	Celery	
Rhizoctonia		
Rhizoctonia solani		
AGs 1, 2.1*, 2.2, 3, 4, 10, 11	Beans	Brown root rot
AGs 2.1*, 2.2, 4, 9	Brassicas	Stem canker
AGs 2.1, 2.2, 3*	Potatoes	Black scurf (tubers), stem canker, wilt (occasionally), tuber canker (occasionally)
AGs 2.1, 8	Onions	Onion Mallee stunt
AGs 1, 4	Lettuce	Bottom rot
AGs 1	Carrot	Carrot black, crown rot
R. crocorum	Carrot	Violet root rot
Rhizoctonia spp.	Cucumber seedlings	Root rot & collar rot

* denotes predominant pathogenic AG

Table 1.2: Control strategies used to manage key soilborne pathogens in Australia

Pathogen	Crop	Predominant control strategy	Other known controls
Sclerotinia minor	Lettuce	Seedling drench – Filan® (boscalid) or Rovral® (iprodione) (mainly Filan®) at transplanting Spray application (low volume) Filan® or Rovral® irrigated in (3 wks after planting) Up to 3 or 4 applications mainly of Filan® only per crop Crop rotation (limited)	Varietal selection (fancy lettuce less susceptible than Cos lettuce) Hydroponics Fumigation (mainly metham sodium) Controlled traffic fixed bed to reduce compaction Oats/green manure break crops Biofumigant crops Wider planting – canopy control Biocontrol – results not good Avoid excessive vigour – smaller heads, less disease. Deep burial (mainly used overseas)
Sclerotinia sclerotiorum	Beans	Two to three sprays with Filan® (boscalid) during flowering Rotation, however, some rotation crops also susceptible (lettuce, potatoes etc)	Calcium sprays (foliar) Irrigation, moisture control, manipulation of fertiliser input around flowering Cereal and pasture break crops Variety selection (less dense) Flowering period (short vs long) – eg. quick petal drop Micro-gypsum sprays
Pythium spp.	Various	Fumigation (metam sodium, capsicum seedling diseases only, carrot, parsnip) Fungicides (metalaxyl - parsley, parsnip, carrot; metalaxyl, phos acid, Aliette® (fosetyl), Previcur® (propamocarb), Terrazole® (etridiazole) – cucumbers but not registered for greenhouse uses) Host resistance (or tolerance) (lettuce, parsley, parsnip, carrot) Climate management (eg. irrigation management for parsley or parsnip or soil temperature management by changing colour of plastic from black to white to reduce soil temperature for capsicum). Rotation (greenhouse cucumber) Hygiene (greenhouse cucumber) Compost/mulch (greenhouse cucumber) Controlling fungal gnats (green house cucumber) Grafting (cucumbers and melons)	Oxygenation of hydroponic solutions. Nutrient and temperature management (hydro lettuce). Biocontrol (hydro lettuce).
Fusarium spp.	Various	Fumigation (mainly Telone® or metam sodium, greenhouse crops, field tomatoes and rockmelon) Fungicide seed dressing (mainly Thiram, peas, beans, snow peas(in trials only)) Moving to new land (snow peas) Grafting (cucumbers, snake beans, tomatoes, melons) Host resistance (or tolerance) (cucumber) Water source treatment (greenhouse crops) Rotation (cucumbers, tomatoes) Hygiene (cucumbers, tomatoes) Delayed sowing (near wilt of snow peas)	Removal of leaves and/or infected plants (cucumber)
Rhizoctonia spp.	Potato*	Seed management potatoes eg. certification, UV treatment for increased vigour, sprouting, chemical treatment including Maxim®	Soil health and management of soilborne inoculum (cultural controls, composts, nutrients, tillage)

		(fludioxonil), Monceren® (Pencycuron) In field chemical treatment potatoes eg. Amistar® (azoxystrobin), PCNB, Rovral® (iprodione), Rhizolex® (tolclofos-methyl) Paddock preparation and selection (weed management, diagnostics, thresholds) Crop rotation	Crop management (planting depth, temperature, nutrition, minimising wounding) Minimising crop stress (eg. reducing windblasting) Cultivar resistance

* Rhizoctonia spp.cause disease in many vegetable crops but predominant control strategies relate to potato. Few controls in place for other crops.

1.2 Current fungicides and their modes of action

Fungicide use in the vegetable industry is complicated by the large number of 'minor' crops for which registrations or permits are required and the lack of national consistency in regulations regarding acceptable 'off-label' chemical use. It would therefore be impossible to accurately detail the effective, available, registered products for each of the four target pathogens in the range of host crops they affect in each state. Instead, this part of the review seeks to highlight generic chemical groups based on their mode of activity and to identify products with a registration for use against one of the four target pathogens anywhere in Australia at the time the review was produced (2008). The review seeks to highlight chemical groups with known activity against the pathogens as a starting point for further work. In many instances the registered use may be in ornamental crops, turf grasses, as a seed dressing, as a mixture with other actives or restricted only to one vegetable crop. For this reason, and because the available information changes constantly as registrations or permits expire, products are withdrawn or new uses are registered, growers must seek accurate, up to date information before considering using any product. This information is available for Australian growers on the Australian Pesticides and Veterinary Medicines Authority website through the Public Chemical Registration Information System (PUBCRIS) http://services.apvma.gov.au/PubcrisWebClient/welcome.do (for registered product searches) or the Agricultural and Veterinary Permits search engine http://www.apvma.gov.au/permits/search.php (for minor use and emergency permits).

1.2.1 Multi site (Protectant) fungicides

Fungicides are metabolic inhibitors. They can be grouped broadly into two types, the older type protectant fungicides and the specific type fungicides. Further classification based on the mode of action of each fungicide has been developed mainly for the purpose of resistance management. Several systems are in place including the numeric Fungicide Resistance Action Committee's (FRAC) code and the fungicide groups (A-X) which are more commonly used in Australia.

Protectant fungicides include copper and sulphur-based products and chemicals such as mancozeb, zineb and thiram. Protective fungicides form a protective film on the plant surface and inhibit germination of the fungal spores. These products are not systemic and will not eradicate infection once established (Stovold 2006). These products have multiple sites of activity on the fungus and the development of resistance is not a serious problem. They are generally applied at high rates, therefore residues, phytotoxicity, environmental effects and chronic low dose health effects can be of concern. In spite of these issues, several of these fungicides remain a useful part of crop protection programs, and are important in programs to reduce the development of fungicide resistance (Dutkey). Protectant fungicides with multi-site contact activity are classed as group Y. The following have registrations in Australia: captan, a phthalimide (for *Pythium, Sclerotinia* and *Rhizoctonia*); chlorothalonil, a phthalonitrile (for *Fusarium* and *Rhizoctonia*); copper oxychloride, an inorganic compound (for *Fusarium* and *Pythium*); guazatine, a guanidine (for *Fusarium*) and the dithiocarbamates mancozeb (for *Fusarium, Pythium* and *Rhizoctonia*), metiram (for *Fusarium*), thiram (for *Fusarium, Pythium, Sclerotinia* and *Rhizoctonia*).

In contrast the specific type fungicides, as the name implies, act on one specific chemical reaction in the fungus. These products have limited protective ability and are instead curative and mostly systemic in activity. These products are applied at relatively low rates, therefore, most leave little if any detectable residue in plant parts or in the environment. They do not kill spores on plant surfaces and, since they are active only at one 'specific site', resistance can become a problem.

In addition to this broad grouping, fungicides have been grouped, mainly for purposes of resistance management, by their mode of activity (FRAC 2007). Those groups with activity against the soilborne pathogen targets (*Sclerotinia, Rhizoctonia, Pythium* and *Fusarium*) are discussed below. Products with registrations in Australia are mentioned if the product is registered for use against the target pathogen genus in at least one crop. This has been done to highlight products that may be worth considering for evaluation in vegetable crop trials. This does not refer to a blanket registration for use against the pathogen in any crop. Potential users should consult the Australian Pesticides and Veterinary Medicines Authority website for current information on chemical registrations and minor use permits in specific vegetable crops (APVMA).

1.2.2 Inhibitors of nucleic acid synthesis

Synthesis of nucleic acid is a vital process for pathogen growth and replication. The group of fungicides that inhibit nucleic acid synthesis includes the phenylamide (PA) fungicides (group D). The target site for the (PA) fungicides is RNA polymerase I. Within the PA chemical group several of the acylalanines have registration in Australia. These include furalaxyl for *Pythium*, metalaxyl for *Fusarium, Pythium* and *Sclerotinia,* and metalaxyl-M for *Fusarium* and under permit for *Pythium* on parsley and lettuce. Resistance and cross resistance towards the PA chemicals is well known in various oomycetes including many species of *Pythium* in carrot (White *et al.* 1988) and ornamental crops (Moorman *et al.* 2002b). This group of fungicides is considered at high risk of resistance developing (FRAC 2007) and of enhanced biodegradation (Al-Sa'di *et al.* 2008; Bailey and Coffey 1985).

1.2.3 Inhibitors of mitosis and cell division

The ability of cells to replicate and divide is fundamental to the ability of the pathogen to survive and multiply within the host. The group of fungicides that inhibit the process of mitosis and cell division includes the Methyl Benzimidazole Carbamate (MBC) fungicides (group A). This group includes two chemical groups, the benzimidazoles (carbendazim for *Fusarium* and thiabendazole for *Fusarium, Pythium* and *Sclerotinia*) and the thiophanates (thiophanate-methyl for *Pythium*) which have registrations for use in Australia. Both of these chemical groups target β-tubulin assembly during mitosis. Resistance brought about through target site mutations in the β-tubulin gene are common and these chemical groups are considered to be at high risk of resistance developing (FRAC 2007). Resistance has been reported in *Fusarium* in solanaceous (Hanson *et al.* 1996; Thanassoulopoulos *et al.* 1970; Tivoli *et al.* 1986) and cucurbit (Bastels-Schooley and MacNeil 1971) vegetable crops, also several ornamental crops (Magie and Wilfret 1974; Valaskova 1983). Resistance has also been reported in *Sclerotinia* in stone fruit (Whan 1976) and turf grass (Cole *et al.* 1974; Detweiler *et al.* 1983; Wong 2003). Cross resistance between chemicals within the group is known.

1.2.4 Inhibitors of respiration

The process of deriving energy from food molecules occurs in the mitochondria. Several fungicides act by disrupting this process in fungal mitochondria. Inhibitors of mitochondrial respiration include the carboxamides (group G). Of this group, flutolanil, a phenyl-benzamide, is registered for Rhizoctonia in Australia (potatoes), and boscalid, a pyridine-carboxamide, is registered for Sclerotinia in Australia with permits covering current uses on alliums, carrots, peas and snow peas against Sclerotinia. These fungicides target complex II in the electron transport chain within the inner membranes of the mitochondria inhibiting succinate dehydrogenase. Resistance to the carboxamides brought about by target site mutations in the sdh gene is known for several fungal species (FRAC 2006).

The strobilurins (Group K) also known as the QoI fungicides (Quinone outside Inhibitors) inhibit respiration. However, this group of chemicals target complex III blocking the cytochrome bc 1 complex (ubiquinol oxidase at the Qo site) (Matheron 2001). In Australia azoxystrobin is registered for use against *Fusarium, Pythium* and *Sclerotinia*. Permits currently cover uses on carrot against *Sclerotinia* and on peanuts against *Rhizoctonia* and *Sclerotinia*. Resistance brought about through target site mutations in the cyt b gene and other mechanisms, is known in various fungal species including *Pythium aphanidermatum* (Gisi *et al.* 2002; Olaya *et al.* 2003). Cross resistance between all members of the QoI group has been demonstrated and this group carries a high resistance risk (FRAC 2007).

1.2.5 Inhibitors of amino acid and protein synthesis

The anilino-pyrimidine (AP) fungicides (group I) including cyprodinil, registered in Australia for Sclerotinia, inhibit amino acid (likely methionine) biosynthesis. Resistance is known (FRAC 2006) and these fungicides are considered at medium risk of developing resistance (FRAC 2007).

1.2.6 Disruption of signal transduction

Those fungicides which disrupt the process of signal transduction in the fungal cell include the phenylpyrrole (PP) fungicides (group L) of which fludioxonil is registered in Australia against *Fusarium, Pythium* and *Sclerotinia*. Resistance to this group has only been reported sporadically and this group is therefore considered at low to medium risk of resistance (FRAC 2007). The target site for this group of chemicals is MAP/histidine kinase in osmotic signal transduction. The dicarboximides (group B) also target MAP/histidine kinase using a different mechanism. Of this group of fungicides iprodione is registered in Australia for use against *Fusarium, Sclerotinia* and *Rhizoctonia.* Numerous permits currently cover its use in specific vegetable crops (eg. beetroot, beans, brassica leafy vegetables, silverbeet and spinach for *Sclerotinia;* and broccoli, cauliflower and cabbage for *Rhizoctonia*). Procymidone is also registered for use against *Sclerotinia* and its use in adzuki beans, culinary herbs, beans and peppers for *Sclerotinia* is currently covered by permit. Recently however, the APVMA recently suspended the registration of this fungicide. This is of significant concern to lettuce and green bean growers who have depended upon it to control lettuce drop and white mould in these crops. The dicarboximides are considered at medium to high risk of resistance (FRAC 2007). Resistance has been reported in *Sclerotinia minor* on lettuce (Hubbard *et al.* 1997) and peanut (Brenneman *et al.* 1987; Smith *et al.* 1995).

1.2.7 Inhibitors of lipids and membrane synthesis

Lipids are an essential component of cell membranes. A large number of fungicides are classified as inhibitors of lipid and membrane synthesis, but, the mode of action of many of these fungicides is, at this stage, proposed rather than defined. This might explain why several of these fungicides are currently classed group X (unspecified mode of action) or group Y (multi-site activity). Of this group of fungicides the aromatic hydrocarbons quintozene (group Y, registered for *Fusarium, Pythium, Sclerotinia* and *Rhizoctonia*) and tolclofos-methyl (group X, registered for *Rhizoctonia*) and the heteroaromatic etridiazole (group X, registered for *Pythium*) are believed to target lipid peroxidation (FRAC 2007). Resistance to PCNB has been reported in *Rhizoctonia solani in-vitro* (Anilkumar and Pandourange Gowda 1981) and *Sclerotium rolfsii* in peanuts (Shim *et al.* 1998). These groups are considered low to medium risk of resistance developing (FRAC 2007).

The carbamates including propamocarb (group Y, registered for *Pythium*) are believed to target fatty acids and affect cell membrane permeability (FRAC 2007). A number of species of *Pythium* exhibit resistance to propamocarb in the USA (Moorman *et al.* 2002a; Moorman and Kim 2004). The group is considered of low to medium risk of resistance developing (FRAC 2007).

1.2.8 Inhibition of sterol synthesis

The most well known and numerous of the sterol biosynthesis inhibitors are the demethylation inhibitors (DMI fungicides, group C). As the name suggests, these fungicides inhibit demethylation in cell membranes targeting C14-demethylase in sterol biosynthesis (FRAC 2007). Of this group the following triazoles are registered in Australia: bitertanol and triadimenol (for *Fusarium* and *Sclerotinia*), difenoconazole (for *Pythium*) and propiconazole and tebuconazole (for *Sclerotinia*). The imidazoles imazalil (registered in Australia for *Fusarium*) and prochloraz (registered in Australia for *Fusarium* and *Sclerotinia*) are also DMI fungicides. Resistance has been demonstrated for various fungal species (FRAC 2006) and cross resistance is generally thought to be present between DMI fungicides active against the same fungus (FRAC 2007). The group is considered of medium risk of resistance developing (FRAC 2007).

1.2.9 Unknown mode of action

The mode of action of some fungicides is not known. These fungicides are classed group X. This group is constantly changing as more information becomes available. Currently the phosphonates including Fosetyl-Al and phosphorous acid (for Pythium) are the only group of chemicals with

registrations in Australia for the target pathogens that fall into group X. This group is considered of low risk of resistance developing (FRAC 2007).

1.3 Chemical control of soilborne plant pathogens in Australia

Application of chemical products (as fungicides or fumigants) is the predominant means of controlling soilborne diseases in Australian vegetable crops. Fumigant use is widespread in the industry with metham sodium being used to manage several *Pythium* diseases (Table 1.2) and Telone™ used for several diseases caused by *Fusarium* spp. (Table 1.2). Effective control of lettuce drop using metham sodium has also been demonstrated in some situations, however, in others no control was achieved. Enhanced bio-degradation of metham sodium was linked to this loss of effectiveness (Wright 2003). Recently the soil fumigant dimethyl disulphide (DMDS) has been shown to destroy 90 % of dormant resting structures of various pathogens including *Sclerotinia, Rhizoctonia* and *Phytophthora* species (Charles 2006).

Sclerotinia has traditionally been managed with fungicide applications when weather conditions are most favourable for disease development. In Australia, chemical control of this disease is currently achieved with the fungicide Filan® (a.i. boscalid). However, there have been reports of unsatisfactory control of lettuce drop and bean white mould with boscalid in many farms in southern Australia and in humid climates such as Gympie, Qld. It is unknown whether these failures are due to inappropriate application methods or time of application. However, it is well known that under conditions of high disease pressure, control of *Sclerotinia* with fungicides is difficult. Recently, resistance to boscalid was detected in field isolates of *Alternaria alternata* from Pistachio in California (Avenot and Michailides 2007, D. Wilson pers. comm.). This highlights the need for research to find alternative fungicides for Filan® to ensure resistance to boscalid does not develop rapidly in populations of *Sclerotinia* in Victoria. Sumisclex® (a.i. procymidone) provided good levels of Sclerotinia control in lettuce and bean crops under Australian conditions before its registration was suspended by the Australian Pesticides and Veterinary and Medical Association (APVMA). The use of this fungicide is currently under review by APVMA. Several other fungicides have been tested for their efficacy against lettuce drop, caused by *S. minor,* but only Bavistin® (a.i. carbendazim) showed promise for *Sclerotinia* control (Pung *et al.* 2005; Villalta *et al.* 2004; Wright 2007). Other fungicides reported overseas to have good activity against *Sclerotinia* diseases of bean and other susceptible crops included Switch® (a.i. cyprodinil + fludioxonil), Folicur® (a.i. tebuconazole) and Shirlan® (a.i. fluazinam). From these Folicur® had a minor use permit for lettuce in Australia, which expires on 30th August 2009. This product has since been registered for control of *Sclerotinia* rot of lettuce (registration due to expire 30th June 2011).

Currently worldwide there are no new fungicides specifically developed for the control of *Sclerotinia* diseases. In Australia in recent years, there have been no new registrations of fungicides for controlling *Sclerotinia* on vegetables (lettuce and green beans), despite the identification of a few efficacious fungicide treatments by various projects funded by Horticulture Australia Ltd (e.g. VG96084, VG00048, VG01096, OT03001). Filan® is the only one which has been widely used under the minor permit user program. A current Tasmanian project VG05090, funded by Horticulture Australia with VC from Nufarm, is investigating the efficacy of new fungicide products for Sclerotinia control as well as liaising with other chemical companies to collect the required efficacy and residue data required for new fungicide registrations. Results from project VG05090 have confirmed that boscalid is very effective at controlling *Sclerotinia* (Hoong Pung, Peracto *pers. comm.).* However, there are no reports of other new fungicides in the pipeline that could be used in the near future for *Sclerotinia* control. There may be some experimental fungicide actives that have potential but these are a long way from commercial use. Consequently project VG05090 is concentrating on boscalid residue trials on a variety of minor vegetable crops.

Screening of fungicides for efficacy against *Rhizoctonia* AG2.1 causing damping-off on green beans is underway in Tasmania (VG05090). To date nine fungicides have been screened in a pot trial. Rhizolex®, Switch®, Tilt®, Filan® and phosphorous acid were shown to inhibit *Rhizoctonia* AG2.1 and significantly improved seedling survival from 16 % in the untreated control to between 41 and 54 % (Pung 2007). A current project (VG06018, led by Barbara Hall, SARDI) is investigating seed, soil and plant treatments to manage brassica stem canker caused by a complex of pathogens including *Rhizoctonia.*

Lack of registrations in some crops and for specific uses (eg greenhouse) and chemical resistance are significant issues for growers managing *Fusarium* diseases. For example, economical control of Fusarium wilt of cucumber can be achieved with chemical drenches of benomyl, however, this chemical was recently withdrawn from sale in Australia (Forsyth *et al.* 2007a). Chemicals are almost completely ineffective in the management of Fusarium wilt of snow pea due to resistance (Ameera Yousiph, Sydney University *pers. comm.*). Recent trial work has demonstrated a significant reduction in Fusarium wilt of cucumber following drench application of Amistar®, Benlate® or Octave® (Forsyth *et al.* 2007a).

1.4 Cultural practices

1.4.1 Crop hygiene

Farm and nursery hygiene should always form the first line of defence against soilborne pathogens. All soilborne pathogens can be transported on or in anything that may carry contaminated soil or water including machinery and equipment, farm workers, livestock, seedling trays, irrigation run off etc.

The effective rates and contact times for a range of disinfectant products and pathogens has been established by a number of researchers and these, together with other methods such as steam sterilisation of seedling trays, are available to disinfect equipment. Waste disposal, removal of infected leaves and/or plants and a range of other general crop hygiene procedures are important to minimise spread of disease particularly within greenhouse crops (Forsyth and Tesoriero, NSW DPI *pers. comm.*). The importance of crop hygiene is being promoted to growers through current HAL funded greenhouse projects (VG04012 and VG05084 led by Len Tesoriero, NSW DPI). This project is also evaluating disinfection techniques for recirculated nutrients solutions (used by hydroponic growers) based on ultrasonics and filtration.

1.4.2 Crop rotation and planting sequence

Crop rotation is an ancient and widely practiced method of disease management. Traditionally cultivation of a non-host crop has been used to prevent the build up of inoculum in soils and to allow time for the natural decay of existing soil inoculum. The benefits, in terms of increased yield and reduced levels of disease in subsequent crops have been recognised for hundreds of years.

More recently, crop rotation has been viewed as a means to increase the biological components in soils that are responsible for disease suppression. In a study of the effects of 62 different 'preceding' crops on the development of fusarium wilt of cucumber and diakon, Murakami *et al.* (2003) report that spinach and burdock significantly inhibited vessel disease of cucumber caused by *F. oxysporum* f. sp. *cucumerinum* (FOC) and that turnip and spinach suppressed yellows of diakon caused by *F. oxysporum* f. sp. *raphani* (FOR). None of the preceding crops significantly reduced the amount of either FOC or FOR recovered from the soil, nor did they affect the production or germination of conidia. In both experiments cultivation of preceding plants increased the number of bacteria in the soil. Additionally, in the FOR experiment actinomycete numbers increased and FOR decreased as a proportion of the total *Fusarium oxysporum* population. It was proposed that these changes in the soil microbial populations were responsible for the observed disease control, rather than any direct effect of the preceding crops on either pathogen.

In the Salinas Valley, California, the inclusion of broccoli as a rotation crop controlled two sclerotial pathogens, *Verticillium dahliae* causing Verticillium wilt of cauliflower and strawberry and *Sclerotinia minor* causing lettuce drop (Subbarao 2004). In this study broccoli specifically increased the populations of actinomycetes and bacteria by 100 and 1000-fold respectively, compared with cauliflower residue. These authors suggest that these actinomycetes and bacteria colonise and destroy microsclerotia, since the observed reductions in microsclerotia numbers remained low for at least 5 years compared with the increases observed following chemical fumigation. Likewise, Gil *et al.* (2008) observed an increase in the native populations of the biocontrol agents *Trichoderma* spp. and *Gliocladium* spp. when maize was used as the crop preceeding peanut. However this corresponded to a decrease in root rot due to *Fusarium solani* at only one of the two sites studied.

In Western Australia the incidence and severity of *Pythium sulcatum* infection (cavity spot) was significantly reduced when carrots were planted following one, two or three broccoli (non-host) crops.

Seedling infection was also reduced, with less forking and increased root length at harvest resulting in an increase in the proportion of export market quality carrots (Davison and McKay 2003). The broccoli used in Western Australian trials contained 4-methylthiobutenyl- and 2-phenylethyl-isothoicynate in their roots. These molecules were not present in the plant leaves. Mycelial growth of *P. sulcatum* was very sensitive to 2-phenylethyl-isothoicynate (Smith and Kirkegaard 2002). It is likely that the release of this molecule from the roots of broccoli crops inhibited the growth of adjacent *P. sulcatum* hyphae.

1.4.3 Planting date and crop density

Modification of the planting date is a strategy often adopted by growers faced with a significant disease problem. For example, anecdotal evidence suggests that some growers will avoid summer plantings of brassicsa to avoid clubroot disease. Delayed sowing is also practiced by some growers in the cotton and snow pea industries to avoid conditions that favour diseases caused by *Fusarium* sp. (David Nehl, NSW DPI and Ameera Youseph, Sydney University *pers comm.* respectively). Wider plant spacing is also effective at reducing diseases such as *Sclerotinia* which spread easily via plant to plant contact (Hoes and Huang 1985).

1.4.4 Tillage and residue management

Traditionally growers have been advised to bury crop residues as quickly as possible after harvest to eliminate them as a potential source of inoculum in subsequent crops. Over many years this practice resulted in significant erosion due to wind and water and reduced soil organic matter by exposing the organic matter in the soil to oxidation (Bockus and Shroyer 1998). Much of the research into the effects of tillage and residue management has been conducted on cereal crops and it has only been relatively recently that reduced tillage and residue management have been considered in Australia as a means to increase the sustainability of vegetable farming systems (Stirling 2008; Stirling and Eden 2008).

In addition to controlling soil erosion and increasing soil organic matter, conservation tillage (leaving 30 % or more of the soil surface covered by crop residue after planting) reduces fuel consumption and soil moisture losses and improves soil tilth and water holding capacity (Bockus and Shroyer 1998). In spite of the many benefits of conservation tillage, the experiences of the grains industry indicate that whilst some soilborne pathogens may be controlled by this practice, other soilborne pathogens are favoured by conservation tillage and may become more problematic under such conditions. For example, the increased soil moisture available to cereals under conservation tillage inhibits diseases caused by *F. graminearum, F. avenaceum* and *F. culmorum* (Bailey and Duczek 1996). However, increased soil moisture favors cereal diseases caused by *Pythium* spp. (Hering *et al.* 1987) and in undisturbed soils cereal root rots caused by *R. solani* are more severe because the pathogen is able to produce web like mycelium (Bockus and Shroyer 1998).

A novel solution proposed for the vegetable industry in northern parts of the Australia seeks to integrate the sugar cane and vegetable industries (Stirling 2008). The proposed farming system integrates minimum tillage and residue retention with crop rotation by planting a vegetable crop directly into sugarcane trash. Sugarcane grows on a 5 or 6 year cycle, and usually follows a legume crop so these soils are likely to have relatively small populations of pathogens specific to vegetable crops. Pathogens such as *Pythium myriotylum* and root-knot nematode, although present in sugarcane soils (Stirling 2008), have been shown to be less problematic in mulched compared to conventional vegetable cropping systems (Stirling and Eden 2008). Stirling (2008) concedes that there are several practical issues that must be addressed before any such integrated system could be implemented. These include vegetable crop nutrition, appropriate planting configurations and the development of machinery able to plant and lay trickle tape into such a system. Clearly the proposed system is not directly applicable to the southern states, but elements of it, for example integration of different farming systems, controlled traffic, residue retention and minimum tillage are likely to become more mainstream as soil health and sustainability become increasingly important concepts in plant pathology.

1.4.5 Rouging

Rouging, the practice of removing infected plants at an early stage of symptom development, is a simple yet rarely practiced means of reducing the build up of soil inoculum becaused it is seen as too costly. At harvest infected plants contain large numbers of resting propagules of the pathogen which

are usually incorporated back into the soil after harvest. Removal of infected plants at an early stage removes the main source of soilborne inoculum in subsequent crops. This technique was used by Patterson and Grogan (1985) to manage lettuce drop. In their study, removal of infected plants in the first crop reduced sclerotia populations 10-fold and disease incidence by 50 %. After three consecutive years of removals the disease was completely eliminated.

According to Isnaini and Keane (2007) successful implementation of this strategy depends upon: (1) the ability to identify symptoms of the disease before resting propagules are formed, (2) the ability to easily remove the bulk of tissues containing resting propagules (ie. resting propagules from lettuce varieties that form sclerotia predominantly on the heads are easier to remove than those which form significant numbers of sclerotia on the tap root), (3) infections occurring over a limited time frame allowing rouging efforts to be concentrated over a limited time.

This method of disease management is labour intensive and time consuming and the results of efforts in the first year may not be fully realised until several seasons later. For these reasons implementation may be more practical in intensive greenhouse based systems.

1.4.6 Heat and solarisation

Thermal inactivation of pathogens caused by the heating of air beneath a polyethylene sheet has been shown to control diseases caused by a number of soilborne pathogens including *Verticillium* spp, *Pythium* spp, *Fusarium* spp, *Thielaviopsis basicola, Sclerotium rolfsii, Rhizoctonia solani, Plasmodiophora brassicae* (Katan 1980; Katan 1981; Porter 1991; Pullman *et al.* 1981). The efficacy of the treatment has been shown to depend upon achieving lethal time-temperature combinations for a given pathogen (Horiuchi *et al.* 1982; Pullman *et al.* 1981). Soil solarization is therefore best suited to warm climates and has been effective in Israel (Grinstein *et al.* 1979; Jocobsohn *et al.* 1980; Katan *et al.* 1976), California (Pullman *et al.* 1979) and Arizona (Matheron and Porchas 2008). In these regions solarisation can be highly effective. For example, solarisation for a period of one month beginning one week after irrigation reduced the incidence of Fusarium wilt on crisphead, green leaf and romaine lettuce by 96, 97 and 88 % respectively (Matheron and Porchas 2008). Elsewhere in climates less conducive to control by solarisation results have been predictably less consistent. For example, in north-western Italy solarisation reduced the incidence of Fusarium wilt of melon by 82-90 % in three of five trials. In one of the remaining trials wilt was reduced by 43.5% but there was no reduction of disease in the other trial (Tamietti and Valentino 2006).

In Australia cooler climatic conditions limit the efficacy of solarisation and would restrict its use to only a few months over the summer in many of the southern horticultural regions (Porter 1991; Porter and Merriman 1983). This together with the cost of treatment and problems associated with the laying and subsequent disposal of plastic have largely prevented its use. However, northern regions such as Darwin and as far south as Katherine experience high temperatures, constant sunshine and intense solar radiation for most of the year (Duff and Pitkethley 2003). In these regions solarisation has been trialled as a means of sterilising potting medium. *Pythium myriotylum, Phytophthora nicotianae* var. *nicotianae* and *Sclerotium rolfsii* at depths of 10 and 25 cm were eradicated with less than 10 days solarisation treatment (Duff and Barnaart 1992). Duff and Pitkethley (2003) conclude that there is potential for solarisation to be used in field situations in Darwin. In such situations plastic could be left in place, painted and seedlings planted directly through the plastic into treated soil.

1.5 Biological soil disinfestation

Soil disinfestation methods using anaerobic decomposition of organic matter from cover crops have been tested as an ecological and economic alternative to soil fumigation with methyl bromide. Biological soil disinfestation has shown promise for reducing the population of soilborne pathogens such as *Fusarium* spp., *Rhizoctonia* spp. and *Verticillium* spp. (Momma 2008). Its potential for reducing the population of sclerotia of *Sclerotinia* spp. in soil has not been investigated.

1.6 Soil pH

The severity of many soilborne diseases is influenced by soil pH. Perhaps the best known example is the relationship between *Plasmodiophora brassicae,* the cause of clubroot, and soil pH. Managing clubroot by raising soil pH is one of the oldest and most widely practiced methods of control (Karling 1968). Incidence and severity of disease is generally reduced at soil pH 7.2 (Colhoun 1958; Karling 1968) and liming is routinely practiced to increase soil pH and manage this disease (Campbell *et al.* 1985).

Likewise, liming to a target pH of 7.2 or higher (measured in calcium chloride) is recommended to reduce the incidence and severity of cavity spot of carrots caused by *Pythium sulcatum* (Davison and McKay 1999). However, damping off diseases caused by *Pythium* species are generally more severe in moist soils with high soil pH (Lucas *et al.* 1992).

In general low soil pH favours the development of wilt diseases caused by *F. oxysporum* in a number of vegetable crops (Anon ; Zitter 1998). Disease development can be limited by raising soil pH to 6.5-7 and using nitrates rather than ammonia for nitrogenous fertilisation (Anon). In Australian research, application of calcium hydroxide (with soil conditioning polymers) applied to the surface of the soil at a rate of 2.5 t/ha maintained the soil pH above 8.5 and this inhibited germination of sclerotia of *S. minor*, resulting in a significant reduction (85 %) in disease severity. However, this result was not reproducible at all field sites tested (Wright 2003).

Different soil types are known to vary considerably in their response to application of liming materials. The pH of soils with a high buffering capacity, such as those found in Santa Cruz and Monterey counties, California (Welch *et al.* 1976), is difficult to adjust. In other soils termed 'lime non-responsive' by Myers *et al.* (1981), lime failed to control clubroot even where the pH of the soil was increased up to 7.7. Application of lime as a strategy to manage soilborne diseases must always be used cautiously as excessive liming can induce deficiencies of trace elements such as zinc, manganese and iron, or liming may have a deleterious effect on crops used in rotation which may prefer lower soil pH (Davison and McKay 1999).

In addition to the effect on the target pathogen, increasing soil pH also alters the soil microbial population and this can affect the ability of antagonists to inhibit disease. For example the biological component of highly alkaline soils appears to play a significant role in reducing the efficacy of Intercept®, a preparation of the biological control agent *Coniothyrium minitans,* a fungal mycoparasite of *Sclerotinia* species (del Rio *et al.* 2004).

1.7 Mineral Nutrition

A relationship between mineral nutrition and plant disease has been recognised for many decades (Walker 1946). This relationship is so well established for some host-pathogen systems that application of nutrient based products is widely recognised today as a tool for disease management; for instance the use of calcium to manage clubroot of vegetable brassicas (Dixon and Webster 1988). There is however no prescriptive approach that can be broadly applied to guide the use of mineral nutrients for disease management. Their role in the management of a range of plant diseases has recently been extensively reviewed (Datnoff *et al.* 2007).

1.7.1 Nitrogen, phosphorous and potassium (NPK)

Nitrogen is widely used in vegetable production. It is added to vegetable cropping soils mainly as ammonia (urea, manure based products, meat and bone meal etc), ammonium or nitrate based fertilisers and is subject to degradation and utilisation according to the nitrogen cycle (Fig. 1.1). Marschner (1986) proposed that high nitrogen increases the susceptibility of plants to diseases caused by obligate parasites but has the opposite effect on diseases caused by facultative parasites, such as *Fusarium* species, due to the different nutritional requirements of the two types of parasite (ie. factors that support growth and metabolism of host cells and delay senescence also increase resistance to facultative parasites).

Ammonia and nitrous acid are themselves toxic to some plant pathogens (Tenuta and Lazarovits, 2002). Ammonia is highly toxic to certain *Fusarium* species, and nitrite is toxic to *Pythium* and *Phytophthora* (Marschner 1986). In addition, different forms of nitrogen absorbed by the plant are

metabolized differently and this has significant consequences in terms of the susceptibility of the plant to disease (Huber and Watson 1974). For example, NH_4 increases the concentration of asparagine in plant tissues. Growth of *R. solani* on stems, production of infection cushions, penetration and lesion enlargement are all increased as the concentration of asparagine increases. NH_4 increases the production of extracellular macerating enzymes by *R. solani* whilst NO_3^- inhibits these (Huber 1991). Hence, damping-off caused by *R. solani* in a range of vegetable crops is less severe when nitrogen is added to the crop as NO_3^- (reviewed by Huber and Thompson (2007)). The longevity of various forms of nitrogen in the soil is modulated by soil pH with ammonia accumulating at high soil pH and nitrous acid predominating at low soil pH (Tenuta and Lazarovits 2002). Diseases caused by *Fusarium oxysporum* (on asparagus, cabbage, celery, cucumber, capsicum and tomato) and *Rhizoctonia solani* (on bean, pea and potato) are reportedly decreased by NO_3^- fertilisation and alkaline pH (Huber and Thompson 2007).

Phosphorus has an important role in energy storage and transfer in plants through adenosine diphosphate (ADP) and adenosine triphosphate (ATP). Phosphorus is also involved in electron transport in oxidation-reduction reactions and has a regulatory role in the formation and transport of sugars (Prabhu *et al.* 2007a). Application of phosphorus has had inconsistent effects on diseases caused by *Fusarium* species but consistently decreased damping off in cucumber and pea and root rot of soybean caused by *R. solani* (reviewed by Prabhu *et al.* 2007a).

Potassium is the most abundant cation in the cytoplasm of plant cells. It is important for the maintenance of osmotic potential of cells and in plant water regulations (Marschner 1986). In contrast to the effects of nitrogen, potassium generally decreases the susceptibility of host plants to both obligate and facultative parasites (Marschner 1986). The beneficial effect of potassium for management of Fusarium wilt diseases is well established (Walker 1946). In their review of the relationship between potassium and plant disease Prabhu *et al.* (2007b) report that potassium decreased the severity of diseases caused by *F. oxysporum* (in banana, cabbage, celery, cotton, melon, palm, pea and tomato), *Pythium* spp. (in beet and hemp) and *Sclerotinia* spp. (on apricot and pumpkin). The effect of potassium on *R. solani* was less consistent. Potassium decreased disease on hemp and jute but increased it on bean, cowpea and peanut. Varying effects were reported on cotton and potato (Prabhu *et al.* 2007b).

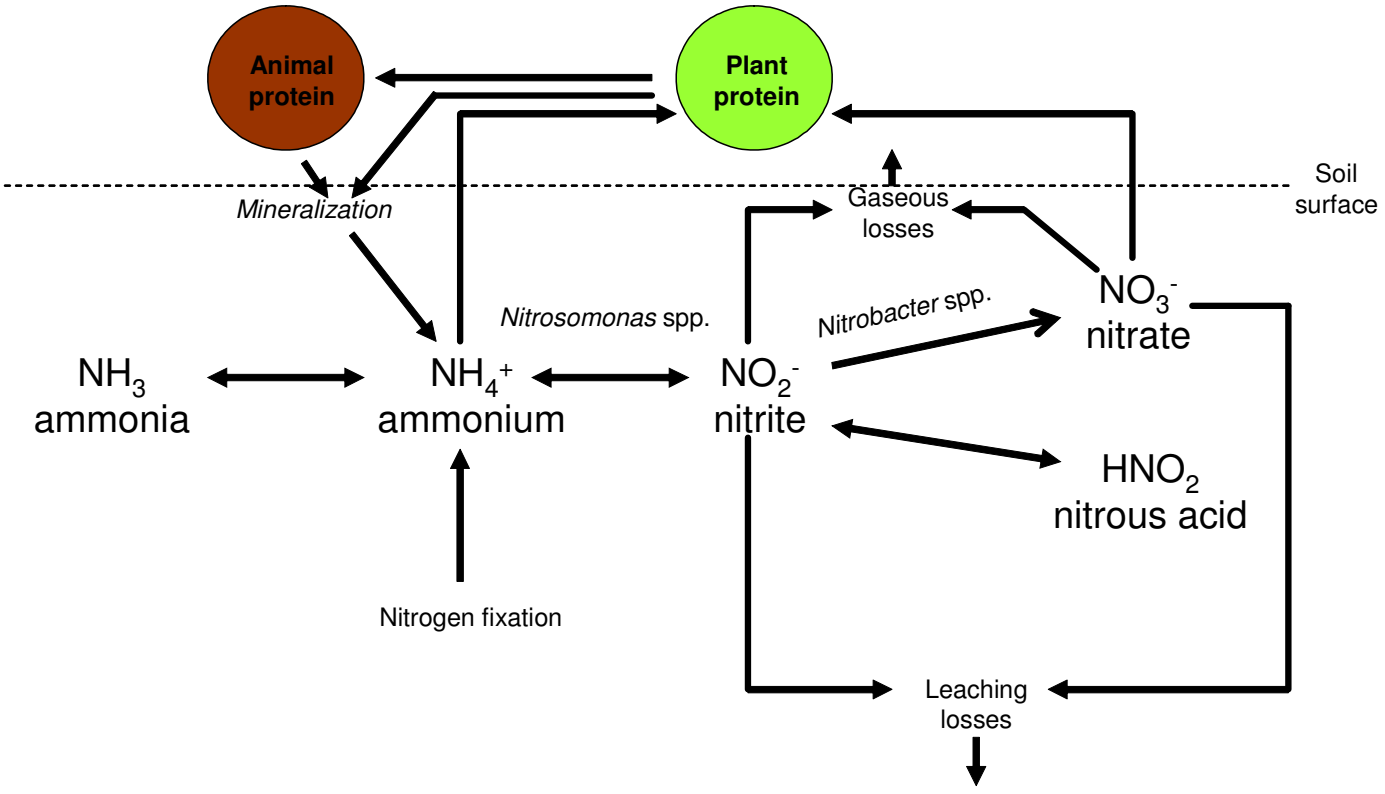


Figure 1.1. The nitrogen cycle (adapted from Huber and Thompson 2007)

1.7.2 Other important macronutrients

After nitrogen, calcium is likely to be the most important nutrient in the management of plant diseases (Rahman and Punja 2007). Application of calcium reduces the severity of cavity spot of carrot and root rot of cucumber caused by *P. coloratum* and *P. splendens* respectively, Fusarium wilt of tomato, peanut pod rot caused by *P. myriotylum* and *R. solani,* and southern blight of carrot and twin stem of soybean caused by various *Sclerotium* spp. (reviewed by Rahman and Punja 2007). A direct effect of calcium on the viability of resting spores of *P. brassicae* has been established for high (1 M) calcium concentrations (Myers and Campbell 1985). Calcium also reduces root hair infection and inhibits the production of differentiated and dehisced sporangia of *P. brassicae* within infected root hairs (Webster and Dixon 1991b).

Calcium is commonly the major cation of the middle lamella of cell walls, where it is bound to polygalacturonic acids (pectins) as calcium pectate and is essential for the structural strength of cell walls. It has been proposed that calcium pectate increases tolerance to the action of cell wall degrading enzymes and may be responsible for the observed reduction in the severity of disease caused by *S. rolfsii* in carrots (Punja *et al.* 1986). Deficient cells cease growing, become disorganised and discoloured and under severe deficiency die (Epstein 1972). Calcium provides this structural strength by cross-linking the pectic chains of the middle lamella (Marschner 1986). Calcium is also essential for the normal membrane structure (by crosslinking of phospholipids and proteins), transport and retention of ions. When calcium is limiting, low molecular weight compounds move from the cytoplasm into the apoplasm. These effects on cell wall and membrane stability are likely to protect the plant from invasion and spread of pathogenic fungi. Corden (1965) reports that resistance to Fusarium wilt in tomato is conferred by a high concentration of calcium in the xylem exudate.

Zoospore motility and chemotaxis play a critical role in the early stages of infection by the soilborne Plasmodiophorid (*Plasmodiophora brassicae* and *Spongospora subterranea*) and oomycete pathogens (*Pythium* and *Phytophthora* species). Potentially, a role exists for calcium regulation of both these processes. Studies using *Phytophthora* indicate that zoospore motility is reduced in calcium deficient media (Cho and Fuller 1989). A similar effect has also been reported for *Pythium porphyrae* (Addepalli and Fujita 2002). Immunolabelling studies have shown high concentrations of the calcium binding proteins, calmodulin (Gubler *et al.* 1990) and centrin (Hardham 1992) within *Phytophthora* flagellae. Calmodulin is distributed differently in the two flagellae, which may elicit a differential response to changes in external calcium, thereby governing directional movement (Gubler *et al.* 1990). Potentially, high levels of calcium could impair flagellar motion and the movement of zoospores towards the host root.

In Australia pre-plant soil treatment with calcium as calcium cyanamide (Perlka™) has effectively reduced the incidence of lettuce drop caused by *S. minor* (Wright 2007) and clubroot of crucifers caused by *Plasmodiophora brassicae* (Donald *et al.* 2004). However, this product has a number of known mechanisms of activity. Perlka™ has a neutralising value of approximately 50 % calcium oxide therefore field applications increase soil pH (Perlka Technical Brochure) and this may directly affect the pathogen. In addition volatiles released from Perlka™ inhibit in vitro growth of some soilborne pathogens including *Rhizoctonia solani, Sclerotium rolfsii* and *Fusarium* spp. (Wicks *et al.* 2001).

Sulphur and sulphur compounds affect disease directly as biocides and indirectly by enhancing plant resistance and increasing the availability of other elements (Haneklaus *et al.* 2007). For example carbon disulphide has been used as a soil fumigant for soilborne fungi, volatile sulphur is used in greenhouses to control powdery mildew and sulphur dioxide is widely used to manage postharvest pathogens in dried foodstuffs (Haneklaus *et al.* 2007). Mechanisms of sulphur induced resistance have been reviewed by Haneklaus *et al.* (2007). These authors propose an essential role for cysteine, an amino acid end product of sulphur assimilation by plants, in induced resistance. As a precursor of coenzyme A cysteine is required for the accumulation of salicylic acid and therefore the production of pathogenesis related proteins via the salicylic acid pathway. Cysteine is also a precursor of glutathionine (part of the antioxidative system of plant cells necessary to prevent damage by reactive oxygen species), many phytoalexins (including low-molecular weight antibiotics) and glucosinolates. Sulphur has not been extensively used to manage soilborne diseases. However, in a recent example soil-applied sulphur significantly reduced stem canker of potato and reduced the rate and severity of black scurf caused by *R. solani* on potato tubers by 41 and 29 % respectively in low sulphur soils (Kilikocka *et al.* 2005).

1.7.3 Micronutrients

Like calcium, boron has long been recognised for its ability to inhibit clubroot and has historically been applied to *P. brassicae*-contaminated soil mainly as borax (O'Brien and Dennis 1936). The mode of action of boron in the *P. brassicae* host-parasite system has been the subject of only a limited number of studies. Using sand solution cultures, Webster and Dixon (1991a) found that, in contrast to calcium and pH (Webster and Dixon 1991b) the effect of boron is not limited only to primary infection within root hairs. Both primary and secondary stages of infection were inhibited by boron. There was also no effect of boron on the number of root hairs infected indicating that the effect of boron is intracellular. In addition to its well documented effects on clubroot disease, application of boron has been shown to reduce the incidence of lesions on beans caused by *F. solani,* wilt diseases of flax and tomato caused by *F. oxysporum* f.sp. *lini* and *lycopersici* respectively, rot and seedling rot of bean, pea and cowpea caused by *R. solani* (reviewed by Stangoulis and Graham 2007).

There are numerous reports of the role of boron in plants, although most have been deduced indirectly, usually from study of boron-deficient plants. Postulated roles for boron include sugar transport, cell wall synthesis, lignification, cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, indole acetic acid metabolism, phenol metabolism and membranes (Marschner 1986). One of the most obvious early changes induced by boron deficiency is the inhibition of elongation of roots and shoots. There are a number of potential roles for boron in the root elongation process, including cell division and cell elongation.

The role of silicon in disease prevention has been extensively studied in rice (Rodrigues and Datnoff 2005). Historically the mechanism of control by silicon has been attributed to a mechanical barrier resulting from the polymerization of silicon *in planta* (Rodrigues and Datnoff 2005). Recently, Si-mediated resistance against pathogens associated with the accumulation of phenolics and phytoalexins and the activation of some PR-genes has been demonstrated (Fauteux *et al.* 2006; Fauteux *et al.* 2005; Rodrigues and Datnoff 2005). A role for silicon, particularly in relation to the control of Pythium diseases is well known although the mode of activity is complex and not fully understood. In cucumber, silicon activates a cascade of biochemical changes associated with specific plant defense reactions including stimulation of chitinase activity and an intense and rapid activation of peroxidases and polyphenoloxidases after infection with *Pythium* spp. (Cherif *et al.* 1994). In addition, these authors reported increased beta-glucosidase activity in silicon treated cucumber roots which correlated with the presence of fungitoxic aglycones in the roots of these plants. This effect on *Pythium* is not universal across a range of host species. Plants differ in their capacity to accumulate silicon. In general monocotyledonss are considered Si accumulators (ie. dry weight of silicon exceeds 1 % of total biomass) and dicotyledons are poor accumulators of silicon. Tomato is a silicon excluder and bitter gourd, a member of the family Cucurbitaceae is a silicon intermediate accumulator. In their study of the infection and spread of *P. aphanidermatum* in these two hosts, Heine *et al.* (2006) report that continuous application of Si significantly inhibited the basipetal spread of the pathogen from the infected root apex in bitter gourd but not tomato. Likewise, silicon has been shown to decrease the severity of Fusarium wilt of cucumber (Bélanger *et al.* 1995; Miyaki and Takahashi 1983) but not tomato (Rodrigues *et al.* 1996).

While most of the studies of responses to silicon fertilisation have been conducted using solution culture, in soils silicon fertilisation has been shown to decrease Fusarium wilt of cucumber (Bélanger *et al.* 1995). However, application of sodium silicate to seedling mix failed to protect cucumber seedlings from damping-off caused by *P. ultimum* indicating that there appears to be a minimum time needed for accumulation of sufficient silicon by seedlings to provide protection against pathogens (Jeffery *et al.* 1997).

An Australian research project VG06009 'Management of vegetable diseases with silicon', (led by Frank Hay TIAR/UTAS) investigated the ability of soluble silicon or silicate fertilisers to reduce various diseases in a range of crops including pea, bean, broccoli and potato. This work demonstrated the ability of potassium silicate to inhibit mycelial growth of several plant pathogens *in vitro,* including *S. sclerotiorum,* and to completely inhibit spore germination of some fungi (Frank Hay, TIAR/UTAS *pers. comm.*). Potassium silicate has also significantly reduced the mortality of cucumber plants due to Fusarium wilt (Forsyth *et al.* 2007a). Likewise, the development of Fusarium wilt disease in banana has been significantly reduced by application of potassium silicate (Forsyth *et al.* 2007b). In the cucumber pot trial however, potassium silicate treated plants died towards the end of the experiment. The treatment was applied only once at the beginning of the experiment. Further experiments with multiple applications are recommended (Forsyth *et al.* 2007a). In Canada potassium silicate amendments have been used in recirculating nutrient solutions to suppress *Pythium ultimum* on long English cucumber (Cherif and Bélanger 1992).

1.8 Organic amendment

Organic amendments such as composts, animal manures and plant residues were commonly used in agriculture to improve soil fertility prior to the availability of chemical fertilisers . More recent evidence suggests that these amendments, often the waste products of other industries, can be used to manage a wide range of soil-borne plant pathogens (Bailey and Lazarovits 2003; Hoitink and Fahy 1986; Hoitink *et al.* 1997).

1.8.1 Composts

In spite of the large volume of literature concerning compost amendments and their use to control soilborne diseases in agricultural systems there are relatively few examples of long term or commercial use of these products for disease control. Compost is a term describing organic matter that has undergone long, thermophilic, aerobic decomposition (Raviv 2008). Composts can be made from a range of organic wastes including sewage sludge, municipal solid waste, animal manures, green wastes and food or industrial wastes. Composts are widely used in agricultural production systems. Observed benefits include improved soil physical properties, slow release nutrients (particularly N, P and micronutrients), soilborne disease suppression, biological weed control, and as an alternative to polyethylene mulch (Ozores-Hampton and Obreza 1998; Stoffella *et al.* 1996). These products are widely used in Australian agricultural systems but they are generally applied for purposes other than disease management. For example, in vegetable production composted poultry manure is routinely used as a source of organic matter and nitrogen and other products such as rice hulls are applied to the soil surface to prevent damage to seedlings that can result from wind erosion of sandy soils.

In a review of the use of composts to control soilborne pathogens Hoitink and Fahy (1986) cite two examples of successful long term use in vegetable production. The first concerns the control of Fusarium brown rot of Chinese yam in the Nagano Valley of Japan using a highly stabilized larch bark compost. In this case application of the compost after fumigation suppressed the development of *Fusarium* spp. by increasing soil populations of *Trichoderma* spp. without changing the total bacteria or actinomycetes (Sekiguchi 1977). In the second example the incidence of lettuce drop caused by *Sclerotinia minor* was reduced in both spring and fall plantings over a four year period by application of composted municipal sludge. In this case disease suppression was correlated with increased microbial activity and changes in N, P, Mg, Ca and total organic matter (Lumsden *et al.* 1982).

The ability of composts to suppress soilborne diseases, particularly damping off and root rot diseases in potting mixes, was first demonstrated by Dr. Harry Hoitink in the late 1970s and early 1980s (Logsdon 1993). Since that time incorporation of composts has been shown to suppress seedling diseases caused by *Sclerotium rolfsii* (dos Santos and Bettiol 2003), *Pythium* (Diánez *et al.* 2005; Pascual *et al.* 2002; Zhang *et al.* 1996) and *Rhizoctonia* species (Kwok *et al.* 1987; Ozores-Hampton and Obreza 1998; Voland and Epstein 1994) and to suppress wilt caused by *Fusarium oxysporum* (Raviv 2008), lettuce drop caused by *Sclerotinia minor* (Lumsden *et al.* 1986) and ashy stem blight of bean caused by *Macrophomina phaseolina* (Ozores-Hampton and Obreza 1998).

There are some reports of near complete disease control following application of composted organic materials (eg. Ozores-Hampton and Obreza 1998). More often the effects are subtle and may be ineffective at high inoculum rates (Voland and Epstein 1994) or may not translate to increased marketable yield (Roe *et al.* 1994). In some instances, application of composted amendments can increase disease severity (eg. sweet corn root rot in Darby *et al.* 2006).

The term ‘compost’ can describe a large range of materials that have undergone thermophilic, aerobic decomposition. The source material and the composting process can be highly variable. This results in enormous variation in the end product and its suppressive ability. For example, van der Gaag *et al.* (2007) studied the suppressive ability of twelve commercially produced green waste composts. None of these induced suppressiveness against *Phytophthora cinnamomi* on lupin, yet three and nine composts significantly induced suppressiveness against *Cylindrocladium spathiphylli* on Spathiphyllum and *Rhizoctonia solani* AG2-1 on cauliflower respectively.

The cause of disease suppression by composts has been extensively studied. It is generally accepted that disease suppression has biotic and abiotic components. Tilston *et al.* (2002) conclude that maturity, formulation and degree of processing are the principle determinants of compost chemistry and in turn of disease suppression. These authors studied composted recycled organic matter and determined that the abiotic component of disease suppression results from the interactions of

extractable organic matter content, nitrogen form and content and pH. They suggested that the size of the contribution made by each of these factors to disease suppression is pathogen specific and may be modified by microbial activity. In addition the effect of each of these individual components on the pathogen may be direct or indirect. High pH (greater than 7.0) for example is directly inhibitory to *Plasmodiophora brassicae* (Murakami *et al.* 2002). At low pH, however, the suppressive effect of a pine bark-glycerine based amendment on *R. solani* was indirectly due to increased populations of *Trichoderma* and *Penicillium* spp. (Huang and Kuhlman 1991).

In many instances total microbial activity is increased in compost amended soils (Darby *et al.* 2006; dos Santos and Bettiol 2003; Pascual *et al.* 2002). In addition, the populations of specific antagonistic microorganisms such as *Trichoderma* spp. (Lumsden *et al.* 1986; Pascual *et al.* 2002), fluorescent pseudomonads (Pascual *et al.* 2002) and *Streptomyces* spp. (Lumsden *et al.* 1986) may also be increased. The suppressive effect of many types of compost can be negated by heating. This is often cited as evidence of the importance of their biotic or microbiological component (Craft and Nelson 1996; Ringer *et al.* 1997). However, a range of non-biotic properties of composts can also influence disease. For example, autoclaving induces a range of changes to compost chemistry including increased extractable carbon and ammonia-N and decreased nitrate-N (Tilston *et al.* 2002). Changes to compost chemistry can also be attributed to the carbon and nutrient sources provided and are responsible for suppressiveness either directly; for example low levels of nitrate-N have been shown to favour Fusarium wilt (Hointink *et al.* 1991), or indirectly; for example by increasing populations of *Trichoderma* and fluorescent pseudomonads (Pascual *et al.* 2002).

Chemical inhibitors of plant pathogens occur in some composts. For example bark or sawdust composts can release compounds such as ethyl esters of hydroxyl-oleic acids that inhibit production of sporangia and zoospores by *Phytophthora* spp. (Hoitink and Fahy 1986). Likewise, DeVleeschauwer *et al.,* (1981) reported that concentrations of the volatile fatty acids (acetic, propionic, isobutyric, butyric and isovaleric) present in fresh compost were above or at inhibitory levels for up to four months following application.

Disease suppressiveness of a compost is rarely due to one single factor. Suppressiveness is due instead to a suite of biotic and abiotic factors. These are determined by the compost maturity, composting process and particle size. Their effectiveness in the field is further mediated by the pathogen itself and the particular conditions that are conducive to disease. This explains why a compost that suppresses one disease may have no effect against another pathogen (van der Gaag *et al.* 2007). Further, different types of composts are frequently suited to the management of specific pathogens or groups of pathogens. For example, tree bark compost with its high carbon to nitrogen ratio generally suppresses Fusarium wilts. Excess nitrogen generally promotes Fusarium wilts so other composts with low carbon to nitrogen ratios (eg. those from sewage sludge) typically increase the severity of these diseases (Hoitink *et al.* 1997; Sullivan 2004).

Ozores-Hampton *et al.* (1998) detail a number of factors to consider before using composts in vegetable crops. These include detrimental effects on plant growth that can result from immature compost, the potential to increase soluble salts, the difficulty in spreading compost products and the potential to immobilize nitrogen, causing nitrogen deficiency if the carbon:nitrogen ratio of the compost is high. In addition low quality, immature compost contains more readily available carbon than mature compost, which can support saprophytic growth of *Pythium* and *Rhizoctonia* species (Nelson *et al.* 1994).

There is little doubt that application of compost can suppress disease and there are examples of its use commercially. Provided salts and other contaminants (such as heavy metals) are monitored and minimised these products are well suited to IPM based systems. However, the development of compost for disease suppression will require significant regionally based research to optimise the compost production using locally available materials for specific pathogens and climatic/soil conditions. Hoitink *et al.* (1997) suggests three approaches to increasing the suppressiveness of compost: curing the compost for at least four months, incorporation of the compost into the field soil several months before planting and inoculation of the compost with biocontrol agents. There is potential to amend composts with one or several microbial agents to increase the disease-suppressiveness (Nakasaki *et al.* 1998). In field trials conducted in Tasmania, application of compost enriched with *Trichoderma* Td22 caused a 51 % decrease in the level of onion white rot (*Sclerotium cepivorum*). This compared favourably with application of the best available chemical treatment, tebuconazole which provided a 40 % decrease in the level of white rot (Metcalf *et al.* 2004).

1.8.2 High nitrogen soil amendments

The active by-products of a range of high nitrogen organic amendments responsible for pathogen kill and the soil factors that influence their efficacy have been studied extensively by a Canadian

research team (Bailey and Lazarovits 2003). High nitrogen organic amendments such as urea and meat and bone meal kill pathogens through the production of ammonia and/or nitrous acid during their decomposition in soil (Tenuta and Lazarovits 2002). The production and retention of these toxic intermediates in soil is dependant on pH and a range of soil factors with ammonia being produced at high pH and nitrous acid produced at low pH (Tenuta and Lazarovits 2002). Ammonia has been shown to accumulate readily following application of meat and bone meal in sandy soils (sand content >70 %) with low organic carbon (<1.4 %)(Tenuta, 2001 cited in Bailey and Lazarovits, (2003)). In the same study nitrous acid was produced where the soil pH was less than 6 and the rate of nitrification was rapid.

Many manure products, having undergone anaerobic fermentation during settling and storage, produce volatile fatty acids (including acetic, propionic, butyric, isobutyric and valeric acid) which can be toxic to pathogens (Tenuta *et al.* 2002). The efficacy of these products depends upon soil pH with toxicity being highest at low pH (Conn and Lazarovits 2000).

Application of high nitrogen amendments to soil has been shown to kill microsclerotia of *Verticillium dahliae* (Conn and Lazarovits 1999; Tenuta *et al.* 2002; Tenuta and Lazarovits 2002). These products are associated with an increase in populations of soil bacteria and fungi, such as *Trichoderma* in the case of liquid swine manure (Conn and Lazarovits 1999; Lazarovits *et al.* 1999).

In Australia the high nitrogen amendments urea, meat and bone meal and ammonium lignosulfate have been used in field trials to control powdery and common scab of potato caused by *Spongospora subterranea* and *Streptomyces scabies* respectively. Meat and bone meal (20 t/ha) and ammonium lignosulfate (5 t/ha) significantly reduced the incidence and severity of powdery scab on cultivars Russet Burbank and Shepody. However, meat and bone meal also stunted the growth, significantly reduced yield and changed the size distribution of tubers of cultivar Russet Burbank resulting in more large tubers (Crump *et al.* 2007).

As with composts the disease suppressive effects of high nitrogen amendments are dependant upon a range of soil factors. Therefore, efficacy must be established regionally using locally available amendments. Like composts, product application is also difficult, particularly where high product rates are required. Additional issues for the commercial use of these products is the potential for leaching of nitrogen into water courses or ground water, the possibility of nitrate residues in foods and possibly coliform contamination of produce which may occur if manure base products have not been prepared adequately (ie. inadequate composting or fermentation). These issues should be addressed before these amendments are considered commercially.

1.8.3 Green manures and 'biofumigants'

Green manures applied as crop residues, organic mulches or plant tissue products have a range of direct (eg. the 'biofumigation' effect of volatiles) (Smolinska and Horbowicz 1999) and indirect effects (eg. altering the soil temperature, increasing waterholding capacity, increasing soil microbial populations and improved growth of the host) (Asirifi *et al.* 1994; Perez *et al.* 2008; Stirling and Eden 2008) on plant pathogens. In this respect, the most well known and widely researched group of plants are brassicas. Brassica plants release biocidal compounds, mainly volatile isothiocyanates produced during the enzymatic degradation of glucosinolates present in plant cells (Smolinska and Horbowicz 1999). Volatile compounds from *B. nigra* and *B. juncea* inhibit mycelial growth of *F. sambucinum* (Mayton *et al.* 1996). Soil incorporation of vegetable brassica crop species has also eliminated propagules of *F. oxysporum* f.sp. *conglutinans* (Ramirez-Villapudua and Munnecke 1988) and significantly reduced onion white rot caused by *S. cepivorum* (Zavaleta-Mejia and Rojas 1990). Different brassica residues differ in the type and amount of volatile compounds released and this determines their toxicity to soilborne pathogens (Smolinska and Horbowicz, 1999). For example, of the nine cruciferous plants tested by Smolinska and Horbowicz (1999) only *B. juncea* affected the sclerotial viability of *Sclerotinia sclerotiorum* whereas chlamydospores of *F. oxysporum* f.sp. *radicis-lycopersici* were killed by three of the nine plants and were suppressed by a further three. BQ mulch™ is a brassica mulch bred and selected to contain high levels of glucosinolates. This product has been effective against soilborne pathogens in New Zealand (Marsh and Cheah 2001) and Australia (S. Mattner, DPI VIC *Pers comm*).

Non-crucifer mulches have been used mainly for their indirect effects on plant pathogens. For example Stirling and Eden (2008) report enhanced microbial activity, increased numbers of free-living nematodes and decreased populations of root-knot nematode under sugarcane mulch compared to the standard plastic mulch. A sorghum-sudangrass hybrid and buckwheat have also been shown to enhance populations of indigenous soil microorganisms antagonistic to *F. graminearum* in wheat residue (Perez *et al.* 2008).

1.8.4 Other organic amendments

Other types of organic amendments have been used to suppress soilborne diseases. In Australia for example, sawdust and molassas (3 % w/v) were effective in suppressing *Rhizoctonia* AG2.1 and gave significant improvement in seedling emergence and survival. Sawdust completely suppressed *Rhizoctonia* and increased seedling emergence and survival to 68 % and 74 % compared to 5 % and 6 % in the untreated control at 12 and 46 days after sowing respectively. At reduced rates (ie. 1 % and 1.5 % molasses and sawdust 1 %) these amendments only partially suppressed *Rhizoctonia* (Pung 2007). While the high rates used are not practical for commercial purposes, the results demonstrate the importance of organic matter in suppression of Rhizoctonia diseases (Pung 2007).

1.9 Disease resistance/tolerance

1.9.1 Genetic resistance

Ideally genetic resistance would be a key component of any IPM strategy for the management of soilborne diseases as it requires no effort for the grower to implement, leaves no residues and has no adverse environmental effects. Some progress has been made in the development of cultivars resistant to some of the key soilborne pathogens. There are very few companies undertaking vegetable breeding programs in Australia. As a result, new cultivars often have not been screened against Australian pathogen isolates.

Generally two forms of resistance exist; physiological resistance where the plant either is resistant to infection or to the growth and spread of the pathogen in host tissue, and resistance conferred by other physical characteristics such as growth habit that indirectly reduce the susceptibility of the host plant to disease. Bean, safflower, sunflower and soybean cultivars resistant to Sclerotinia diseases are available. Predominantly the form of resistance is physical. For example bean cultivars with upright growth habit, narrow canopy and indeterminant growth habit are less susceptible to *S. sclerotiorum* (Huang and Kemp 1989). Likewise, characteristics such as early maturity confer partial resistance of safflower and soybean to *S. sclerotiorum* (reviewed by Bardin and Huang 2001). Physiological resistance to *S. sclerotiorum* may be characterised by a lower rate of diffusion of pathogen-produced oxalic acid in leaf tissue (beans) or by elevated levels of inhibitors (eg. proteinase inhibitors) in leaf tissue (Konarev *et al.* 1999; Tu 1985). However, Bardin and Huang (2001) report that breeding programs have had limited success at increasing physiological resistance possibly because resistance to *S. sclerotiorum* is governed primarily by additive gene action. In Tasmania twelve cultivars of dry bean from the US, with different plant growth habit (climber and non-climber) and the local cultivar Celtic (green bean) have been evaluated for their resistance to *Sclerotinia sclerotiorum*. Flowering patterns were variable for dry beans and not all cultivars reached the pod stages. Disease incidence levels recorded several weeks after flowering were also variable ranging from 0 to 68 %. The majority of disease symptoms were due to secondary *Sclerotinia* infections from senescing flowers that lodged on stems. The highest levels of susceptibility were associated with more vigorous growth. The disease levels were highly correlated with growth stages and while there were significant differences in the incidence of white mould between the different cultivars, these differences were mainly attributed to differences in growth stages rather than genotypic differences (Villalta and Pung 2004).

Different types of lettuce differ in their susceptibility to Lettuce drop caused by *S. minor* and *S. sclerotiorum,* for example fancy lettuce is less susceptible than Iceberg or Cos lettuce. The upright stature of Cos lettuce suggests that it is less susceptible than iceberg lettuce to leaf-to-leaf spread, in particular. However, anecdotal field observations suggest that when primary infection is high (soil to leaf or crown of plant) then the spread of the disease via leaf-to-leaf contact is high even for Cos lettuce (Oscar Villalta, DPI Victoria, *pers. comm.*).

Resistance to *Fusarium solani* has been reported in cucumber (El-Kazzaz and El-Mougy 2001). In contrast to the current situation in Europe, there is no effective resistance in the cucumber lines currently grown in Australia to *F. oxysporum* f. sp. *cucumerinum*. Resistance exists in Australian grown cucumbers but it is only effective against races 1 and 2, not race 3 of this pathogen (Len Tesoriero, NSW DPI *pers. comm.*). Likewise, whilst resistance to *F. oxysporum* f.sp. *pisi* occurs in pea varieties, there is currently no known resistance in Australian commercial snow pea varieties (Ameera Yousiph, Sydney University, *Pers comm.*.) Andrew Watson, NSW DPI screened snow pea lines for resistance to *Fusarium* wilt in a recent HAL funded projece (VG05029) but did not find any cultivars

resistant to the disease. A resistant line of basil, Nufar F1, was selected in Israel as a line which did not develop symptoms when grown in infested soil. The original material imported from USA tested as susceptible to the disease, developing normal disease symptoms. Nufar F1 was selected from this material over six generations. (Reuveni *et al.* 1998). Northern Territory growers trialled Nufar F1, "UH sweet basil" from Hawaii and "5170" from Rijk Zwaan seeds. They found "UH sweet basil" and "5170" unsuitable. Nufar F1, whilst not totally immune, was more resistant than conventional sweet basil. The market prefers the normal susceptible sweet basil, but will accept Nufar F1 at a lower price. Growers are recommended to destroy any obviously affected "Nufar F1" to reduce inoculum build-up, allowing remaining plants to last longer before they succumb to the disease (Condé *et al.* 2005).

1.9.2 Grafting

Grafting is the uniting of two living plant parts so that they grow as a single plant. Grafting is practiced to improve yields, improve tolerance to salinity or other environmental stresses but it is most commonly practiced to manage soilborne diseases. In this instance a commercially acceptable variety is grafted onto a disease tolerant rootstock. In this way, the high yield and commercial quality of the chosen variety can be maintained in the presence of disease.

There are many reports of grafting being used to manage Fusarium diseases, eg. bean (Cichy *et al.* 2007), snake bean (Condé *et al.* 2002), melon (Cohen *et al.* 2002; Lee 1994) , cucumber (Pavlou *et al.* 2002), watermelon (Paroussi *et al.* 2007) and tomato (Harrison and Burgess 1962). In addition, grafting has been used to control Verticillium wilt (Bletsos 2006; Bletsos *et al.* 2003) and bacterial wilts in eggplant and tomato (Karim *et al.* 2003; Tikoo *et al.* 1979).

Grafting is labour intensive and expensive as two varieties of seed must be purchased and raised. It is therefore generally only suited to low density or high value plantings such as cucurbits, snake bean and some solanaceous crops. Grafting is effective for the management of soilborne pathogens where suitable resistant rootstock exist. Graft incompatibility, the premature death or failure of the grafted plant to grow normally, can result in impaired transport of photosynthates (Stigter 1971) and lead to reduced fruit quality and yield and general failure of the plant to thrive. Even compatible resistant rootstocks must be carefully selected as they can confer additional effects (good and bad) on the grafted scion. In watermelon for example, grafting can affect sugar and lycopene content, fruit firmness and yield (Roberts *et al.* 2007). In snake bean, grafting onto Fusarium wilt resistant iron cowpea also confers resistance to root-knot nematodes (Condé *et al.* 2002). In addition, many soilborne pathogens also have an aerial stage to their lifecycle. Conidia of some *Fusarium* spp. can be airborne (Gamliel *et al.* 1996; Katan *et al.* 1997). Grafting will only provide protection against these species to the extent that failure of the soilborne inoculum to infect the host crop prevents sporulation on these plants and therefore reduces the available airborne inoculum. If alternative sources of airborne inoculum exist (eg. crop debris or adjacent crops), grafting alone will not prevent disease as the above ground parts of the grafted plant remain susceptible to airborne infection.

In spite these problems, grafting is currently being used to control several soilborne pathogens mainly in Asia and developing countries. In 2000, 700 million grafted vegetable crop plants were used in Japan and Korea (Edelstein 2004). In developing countries the technique is gaining popularity as it has been shown to be highly cost effective. In Bangladesh for example, eggplant yield was increased by 254 % using grafting to manage bacterial wilt caused by *Ralstonia solanacearum* (Karim *et al.* 2003). This translated to an increase in income of 299 % over the practice of planting non-grafted eggplants and treating with pesticides. In Australia grafting snake beans onto resistant cowpea rootstock has been used to manage Fusarium wilt in snake beans (Condé *et al.* 2002)

As the cost of chemicals increases and further restrictions are placed on the use of fumigant products such as methyl bromide, grafting is likely to become more cost effective in other parts of the world, particularly in greenhouse industries. Since the control of a particular disease can be achieved without chemicals by grafting, this technique can significantly reduce the chemical input into a crop.

1.10 Surfactants and biosurfactants

Zoosporic plant pathogens are a large and diverse group, including economically important soilborne organisms which are either directly pathogenic (eg. *Phytophthora, Pythium, Plasmodiophora, Spongospora, Aphanomyces*) or act as vectors for viruses (eg. *Olpidium*). All of these microorganisms have in common a unicellular, flagellate stage in their lifecycle. These structures are motile in water and are the means by which the pathogen reaches its host. They have no protective cell wall and are

surrounded instead by plasma membrane. This therefore is potentially the most vulnerable stage of the lifecycle. Surface active agents, including non-ionic surfactants, are known to disrupt cell membranes because they dissolve the lipid membrane. This lowers the surface tension of the membrane allowing water to flow into the cell and resulting in cell lysis (De Jonghe *et al.* 2007). Lysis of *Olpidium brassicae* zoospores has been observed following treatment with anionic, cationic and non-ionic surfactant types (Tomlinson and Faithfull 1979). *In vitro* activity of a non-ionic synthetic surfactant has been demonstrated for *Pythium* and *Phytophthora* species (Stanghellini and Tomlinson 1987). *In vivo* non-ionic surfactants have been used in hydroponic systems to control lettuce big vein and melon necrotic spot of cucumber, both of which are viruses vectored by zoospores of *Olpidium* species (Tomlinson and Faithfull 1980; Tomlinson and Thomas 1986), and root rot of cucumbers, capsicum and witlof chicory caused by *Pythium aphanidermatum*, *Phytophthora capsici* and *Phytophthora cryptogea* respectively (De Jonghe *et al.* 2005; Stanghellini *et al.* 1996a; Stanghellini *et al.* 1996b). In substrate culture a synthetic non-ionic surfactant effectively controlled root and collar rot of tomato caused by *P. nicotianae* (De Jonghe *et al.* 2007). There have been few studies to evaluate the use of surfactants for the control of zoosporic pathogens in potted soil or organic substrates. Stanghellini *et al.* (2000) were able to control *P. capsici* on capsicum in organic substrates but results obtained by De Jonghe *et al.* (2007) for *P. nicotianae* on tomato were extremely variable. These authors thought that this may have been due to adsorption of the surfactants in the potting soil, and suggested that since non-ionic and cationic surfactants have a much higher sorption on soil and sediment than anionic surfactants it may be worthwhile evaluating anionic surfactants for use in soil based systems.

Surfactant products offer an inexpensive alternative to fungicides. Their efficacy in hydroponic systems against zoosporic pathogens appears well established. In Australia some work demonstrating effective management of disease in hydroponic lettuce using Agral™ has been conducted (Len Tesoriero, NSW Department of Primary Industries *Pers. comm.*) but broader investigation of the use of surfactant products in Australian hydroponic systems warrants further investigation. However, it is important to realise that, due to their mode of action being disruption of lipid membranes, the efficacy of these products is likely to be restricted to zoosporic pathogens. Not all *Pythium* species are zoosporic (Plaats-Niterink 1981). In Australia several non-zoosporic *Pythiums* have been identified from vegetable crops (Len Tesoriero, NSW Department of Primary Industries *Pers. comm).*

Surfactant use in soil based growing systems is less well researched. However, evidence from the hydroponic systems is sufficiently convincing to suggest that a study of the efficacy of a range of surfactants in soil based systems, together with a study on the effects of application of these type of products on soil properties is warranted.

Other work that may be of direct relevance to both soil based and hydroponic systems is the use of naturally produced 'biosurfactants'. Biosurfactants are produced by a range of microorganisms that reside in the soil and on plant surfaces including the bacteria *Pseudomonas, Bacillus, Arthrobacter, Rhodococcus, Acinetobacter* and *Corynebacterium* and the fungi *Candida* and *Torulopsis* (Stanghellini and Miller 1997). Biosurfactants have the same essential function as synthetic surfactants (ie. zoospore lysis). Biosurfactant research is progressing rapidly driven mainly by the search for organisms suitable for bioremediation purposes (eg. decontamination of environmental spills). Rhamnolipids (a class of glycolipids produced by *Pseudomonas* spp.) are the best studied biosurfactants. Rhamnolipids have been shown to be zoosporicidal against *Pythium aphanidermatum, Phytophthora capsici* and *Plasmopara lactucae-radicis* (Stanghellini and Miller 1997).

One potential role for biosurfactants in nature is that they confer upon the producing microorganism an ability to utilise organic carbon sources that might otherwise be unavailable. This has been exploited by Colbert *et al.* (1993b) who were able to enhance the population density of an introduced *Pseudomonas* biological control strain in agricultural field soils by selective feeding with salicylate (a low-molecular-weight aromatic compound). Further, this research team were able to transfer the plasmid that conferred the ability to utilize salicylate from *Pseudomonas putida* PpG7 to *P. putida* R20, an antagonist of *Pythium ultimum* (Colbert *et al.* 1993a). Potentially this could increase the competitiveness of biocontrol agents which often perform well in laboratory and glasshouse trials yet fail to establish in field conditions.

1.11 Plant derived compounds

A range of plant derived compounds with anti-fungal or anti-microbial activity have been identified. These consist of two basic categories of plant compound; antioxidants and secondary metabolites

(Brooker *et al.* 2004) including phenolics and polyphenolics, terpene/triterpenoids and essential oils/lipids, alkaloids, lectins and polypeptides (Cowan 1999). The antimicrobial activity of plant products and their potential use in the pharmaceutical industry has been comprehensively reviewed by Cowan (1999). Mechanisms of activity including substrate deprivation (simple phenols and tannins), membrane disruption (simple phenols, tannins, terpenoids and essential oils), binding to adhesins (quinones, flavonoids and tannins), complexing with cell walls (quinones, flavones and tannins), inactivation of enzymes (quinones and flavones), binding to proteins (tannins), metal ion complexation (tannins), intercalation into cell wall and/or DNA (alkaloids) and the formation of disulfide bridges (lectins and polypeptides) are attributed to the major classes of antimicrobial plant products.

Many of these compounds arise from plants that we consider aromatic, for example herbs and spices. Sekine *et al*.(2007) screened volatiles from 52 herbs and spices against *Fusarium oxysporum* reporting that black zira (*Bunium persicum*) showed the strongest effect followed by cumin and cardamom. Analysis of the volatiles found cuminaldehyde and *p*-cymene had the strongest antifungal effect against *F. oxysporum.* In many plant species resistance to pathogens depends upon a higher content of phenolic compounds (Nicholson 1992). In cumin varieties tolerance to *F. oxysporum* f. sp. *cumini* for example, has been shown to be associated with a higher content of salicylic acid and hydroquinone pre-infection and higher umbelliferone content in root tissue at pre and post infection stages (Mandavia *et al.* 2000). Most of the studies of the antifungal properties of plant derived compounds have been conducted *in vitro.* There are relatively few reports of the activity of these compounds against soilborne pathogens in soils. Soil amendment with essential oils from oregano and fennel have been shown to reduce sclerotial viability and mycelial growth of *Sclerotinia sclerotiorum* resulting in increased survival of tomato seedlings by 69.8 and 53.3 % respectively (Soylu *et al.* 2007). In greenhouse studies commercial preparations of botanical extracts of pepper/mustard, cassia and clove applied at 10 % aqueous emulsions reduced the population density of *Fusarium oxysporum* f.sp. *chrysanthemi* by 99.9, 96.1 and 97.5 % respectively. A similar effect was reported against *F. oxysporum* f.sp. *melonis* on muskmelon where these treatments resulted in 80-100 % of healthy plants compared with less than 20 % for the untreated infested controls (Bowers and Locke 2000). Interestingly in this work neem oil extract increased the population density of *F. oxysporum* f.sp. *chrysanthemi* at all rates tested.

Among the best known and most extensively studied plant volatiles are the isothiocyanates released from tissue of cruciferous plants during hydrolysis of glucosinolates, and volatile extracts from garlic (*Allium sativum* L.). The toxicity of crucifer derived isothiocyanates to fungi has been known for many years (Walker *et al.* 1937). Importantly these compounds have been shown to inhibit mycelial growth (eg. *Fusarium sambucinum* Mayton *et al.* 1996) but also to be directly toxic to resting structures, such as chlamydospores of *Fusarium oxysporum* f. sp. *conglutinans* and *Fusarium oxysporum* f.sp. *radicis-lycopersici* (Ramirez-Villapudua and Munnecke 1988; Smolinska and Horbowicz 1999) and sclerotia of *Sclerotium cepivorum* (Smolinska and Horbowicz 1999; Zavaleta-Mejia and Rojas 1990). When tested against the larger sclerotes of *Sclerotinia sclerotiorum,* however, volatile isothiocyanates delayed sclerotial germination by 7 days but did not significantly affect viability (Smolinska and Horbowicz 1999). This was believed to be due to the reduced penetration of the volatiles into the centre of these larger sclerotes. Glucosinolates occur naturally in all cruciferous plants but the types and amount of the compounds present differs significantly. Therefore cruciferous plants should be screened against the pathogen of interest before selection as an organic amendment (Smolinska and Horbowicz 1999).

Like isothiocyanates, the antimicrobial properties of allicin, a compound produced in garlic after tissue damage exposes alliin to alliin-lyase have been known for many years (Cavallito and Bailey 1944). Treatment with garlic extract has been shown to inhibit the mycelial growth of a range of fungi *in vitro* including *Pythium ultimum, Rhizoctonia solani, Fusarium solani* and *Phytophthora infestans* (Bianchi *et al.* 1997; Curtis *et al.* 2004). *In vitro* treatment caused collapse of hyphae of *R. solani* and caused hyphae of *F. solani* to appear thinner and contain more vacuoles than the controls (Bianchi *et al.* 1997). Curtis *et al.* (2004) were also able to demonstrate a reduction in disease caused by *Phytophthora infestans* in potato tubers by application of allicin applied directly at the site of inoculation or by fumigation. This second technique opens up real possibilities for the use of this compound in agricultural systems. There are a number of issues with the use of allicin specifically but plant compounds in general that have slowed the use of these compounds in agricultural systems. The first is the potential for tainting of the harvested produce with an unwanted odour or taste. Of significance for the agricultural application of such compounds is the ability of fungi to recolonise treated areas. Using *in vitro* tests, Curtis *et al.* (2004) observed that some fungi, specifically *Botrytis cinerea,* were readily able to recolonise inhibition zones after a few days further incubation, whereas other fungi were not. Also of concern is the potential for human toxicity. Where the disease problem

occurs in the early stages of seedling growth (ie. damping off and seedling rots) seed treatment represents a low cost method of application that reduces the potential for taint and human toxicity. This method has been shown to be effective for applying a plant derived lipid, sesamol (3,4-methylenedioxy-phenol) and a terpene/triterpenoid phytoalexin analogue for control of fungal rots of soybean caused by *Macrophomina, Phythium, Phytophthora, Phomopsis* and *Rhizoctonia.* In a field trial, the survival of seedlings treated with either of the plant derived seed treatments was at least as high as the commercial fungicide seed treatments (Brooker *et al.* 2004).

1.12 Biological control

Traditional methods used to protect crops from diseases have been based largely around chemical control methods. Application of fumigant and fungicide products can have adverse, sometimes catastrophic (eg. the effects of fumigation with methyl bromide on the ozone layer) environmental impacts and there is increasing awareness that long term use of many of these products is not sustainable. There is also a perception amongst consumers that consumption of chemically treated produce may adversely affect their own health. Increasingly primary producers are coming under pressure from consumers to reduce chemical usage. In addition, some of the chemical products that they have traditionally depended upon are being withdrawn from the marketplace. Recent examples include the phase-out of methyl bromide, a soil fumigant used to control soilborne fungi, and the withdrawal of procymidone for treatment of *Sclerotinia* diseases of vegetable crops (including lettuce, onion and bean). Many growers have become interested in reducing their dependence on chemical inputs. Biological control, used alone or in conjunction with reduced chemical input, offers growers one means to reduce chemical use. Biological control involves the use of beneficial organisms, their genes and/or products such as metabolites, to reduce the negative effects of plant pathogens and promote positive responses by the plant (Vinale *et al.* 2008). Biological control can occur naturally (eg. suppressive soils) or it can result from the addition of biological control agents (BCAs). There is an enormous and growing amount of literature available concerning biological control. This is demonstrated by the developments in the Netherlands cited by van Lenteren in his foreword to the third edition of the manual of biocontrol agents (Copping 2004). This author explains that in the 1970s about 300 active chemical ingredients were available in the Netherlands to produce thousands of chemical pesticides. At this time the first two microorganisms became available as biological control agents. By 2004 the number of active chemical ingredients was reduced to 70, whereas the number of commercially available biological control agents had increased to more than 100 species.

1.12.1 Suppressive soils

Suppressive soils are a natural phenomenon. They occur all over the world for a range of pathogens and are of interest for their inherent value to primary producers and as a potential source of biological control agents. There are two classic forms of suppressive soils. Those exhibiting a general form of suppression are suppressive to a range of plant pathogens. The suppressiveness is brought about by a high total soil microbial biomass which either competes with the pathogens for resources or is antagonistic towards them. No single microorganisim is responsible for the suppressiveness. This form of suppression may be enhanced by the addition of organic matter or other practices which increase soil microbial activity. This form of suppression is not transferable to other soils. By contrast, specific suppression is transferable. It is specific generally to one pathogen or closely related pathogens and it can be caused by a single microorganism, although frequently it is brought about by multiple soil microorganisms. For example, Broadbent *et al.* (1971) examined 60 potentially suppressive Australian soils and found that *Bacillus* spp. and *Streptomyces* spp. were the main microorganisms responsible for inducing suppressiveness to Rhizoctonia and Pythium damping-off.

The microbial basis for specific soil suppressiveness to plant pathogens is reviewed by Weller *et al.* (2002). These authors provide a number of examples of suppressive soils including those which are suppressive to Fusarium wilt caused by *F. oxysporum* and apple replant disease caused by a complex of fungi including *Cylindrocarpon destructans, Phytophthora cactorum, Pythium* spp. and *Rhizoctonia solani.* These examples provide insight into the complexity of the microbial basis for soil suppressiveness and why soil suppressiveness is so difficult to replicate artificially as a tool for managing plant pathogens.

Of the Fusarium wilt suppressive soils reviewed by these authors suppressiveness was either long standing or induced by monoculture. In all cases non-pathogenic *F. oxysporum* were implicated.

At several sites fluorescent *Pseudomonads* were also involved. Mechanisms of suppression included competition and induced systemic resistance.

By contrast, soils that had never grown apples were suppressive to apple replant disease. *Burkholderia cepacia* and *Pseudomonas putida* were associated with suppressiveness to apple replant disease. *B. cepacia* is an antibiotic producing microorganism with biocontrol activity against a range of soilborne pathogens including *R. solani* and *Pythium* spp. (Parke and Gurian-Sherman 2001). It is marketed as Deny® in the USA. *P. putida* was antagonistic to *Pythium* and *Rhizoctonia* spp. and has been patented for the control of apple replant disease (Mazzola 1999). Cultivation of apples decreased the populations of these microorganisms and shifted the soil *Pseudomonas* population in favour of *P. syringae* and *P. fluorescens* biovar C (not inhibitory to the replant pathogens).

Although only a few microorganisms may be responsible for suppressive activity and these can be relatively easy to identify in suppressive soils, these biocontrol agents interact with a suite of other rhizosphere microorganisms. Together with a host of soil, environmental and abiotic factors these regulate the degree of suppressiveness. Therefore introducing large populations of a microorganism conferring disease suppression in one soil to a disease conducive soil will not replicate the rhizosphere microbial community from which that population was isolated. This is the single biggest reason why many introduced biocontrol agents fail to establish and therefore fail to control disease.

A range of molecular methods have recently been developed and are now widely in use to characterise microbial communities of suppressive soils (eg. Hjort *et al.* 2007; Mazzola 2004). These methods provide much greater insight into the microbial structure of suppressive soils and the way in which these are modified by agricultural practices. Hopefully this will provide information to assist growers manage soil microbial communities for enhanced disease suppression. They also offer targeted screening techniques that will enable researchers to rapidly select potential biocontrol agents on the basis of the functional compounds they produce.

1.12.2 Plant Growth Promoting Rhizobacteria (PGPR), biological control agents (BCA) and endophytes

Soilborne plant pathogens must compete with a suite of microorganisms that colonise the soil and root surface. The rhizosphere, the area of soil immediately surrounding the plant root system, in particular, supports a large active microbial population due to the presence of organic root exudates. Plant growth promoting rhizobacteria (PGPR) are rhizosphere inhabitants. These bacteria including members of the genera *Pseudomonas, Bacillus, Serratia* and *Azospirillum* enhance root and plant growth and can also limit the growth of some soilborne pathogens (Kloepper 1991). PGPR which limit the growth of soilborne pathogens are also biological control agents (BCA) however, BCA also include many genera of fungi. PGPR or BCA which are able to colonise host roots without compromising the host are endophytes. Endophytic fungi have been isolated from a range of healthy vegetable crops including cucumber, red pepper, tomato, pumpkin and Chinese cabbage. Several of these exhibit potent *in vivo* anti-oomycete activity (Kim *et al.* 2007). PGPR, BCA and endophytes have a range of potential modes of action. Many of these are reviewed in Compant *et al.* (2005). PGPR have been associated with the synthesis of plant-growth regulators such as IAA, iron chelation by siderophores, biological control of soilborne pathogens and induction of the systemic defense response (Montesinos *et al.* 2002). BCA are predominantly competitors and fungal antagonists. BCA colonize or compete for nutrients and sites of pathogen interaction with plants. They may also exert antagonistic pressure on pathogens including synthesis of antimicrobial compounds (eg. bacteriocins) or antibiotics. BCA may directly interfere with the pathogen or parasitise it (hyperparasitism). Occasionally BCA induce systemic acquired resistance (Montesinos *et al.* 2002; Zhou and Paulitz 1994). Endophytes suppress disease predominantly through induction of systemic acquired resistance, production of enzymes, siderophores, antibiotics or other antifungal substances. Frequently individual biological control agents can have more than one mode of activity. *Trichoderma* species for example are well known mycoparasites. However, *Trichoderma* species also produce a range of antimicrobial secondary metabolites, compete with pathogens for nutrition, space and infection sites and can induce plant defence responses (Vinale *et al.* 2008).

Competitive root colonisation

Root exudates are a source of carbon and the nutrient rich root surface attracts large numbers of microorganisms. Since root exudates are the main source of nutrition for rhizosphere microorganisms, rhizosphere competence implies that PGPR are well adapted to their utilisation (Compant *et al.* 2005).

Root colonisation by BCA or PGPR reduces the availability of nutrients and pathogen entry sites (Montesinos *et al.* 2002).

Mycoparasitism

Some microorganisms are directly parasitic towards plant pathogenic fungi. Amongst the most well known mycoparasites are the *Trichoderma* spp. These BCA produce high molecular weight cell wall degrading enzymes that hydrolyse the cell wall of the host fungus which, in turn, releases low molecular weight oligomers. These degradation products reach the mycoparasite and activate a mycoparasitic gene expression cascade (Vinale *et al.* 2008). Biological control activity of *Trichoderma* spp. has been demonstrated against *R. solani* (Elad *et al.* 1982; Singh and Chand 2006), *S. rolfsii* (Elad *et al.* 1982), *Fusarium oxysporum* f. spp. *radicis lycopersici* and *niveum* (Ozbay *et al.* 2004; Sivan and Chet 1986) and *Pythium ultimum* (Naseby *et al.* 2000) although in each of these cases mycoparasitism is unlikely to be the sole mechanism of activity.

Coniothyrium minitans is another well known mycoparasite of *S. sclerotiorum* (Huang and Kokko 1988; Huang and Kuhlman 1991; Jones and Stewart 2000; McLean *et al.* 2004) and other sclerotia forming species (Whipps and Gerlagh 1992). Recent evidence suggests that the production of antifungal substances by *C. minitans* also plays an important role in the biological control activity of this mycoparasite (Yang *et al,* 2007).

There are several biocontrol options available for the control of Sclerotinia diseases caused by *Sclerotinia sclerotiorum*. However, there are only limited options available for effective control of lettuce drop, caused by *S. minor*. *Coniothyrium minitans* (Contans™), for instance is a commercially available fungal mycoparasite with proven efficacy against *S. sclerotiorum*, but not *S. minor*. Recently, however, this biological control agent was reported to effectively reduce the incidence of lettuce drop, caused by *S. minor* and *S. sclerotiorum* under field conditions in California (Pryor 2007, KV. Subbarao, University of California, Davis and P. Lüth, Prophyta Biologischer Pflanzenschutz, pers. comm). In trials in Holtville California, two applications with the highest rate of Contans tested (10 lb/acre) was equally effective as the fungicide Endura™ (a.i. boscalid) for the control of lettuce drop caused by *S. minor* (Pryor 2007). In the same trials, the two rates of Contans tested (2 and 10 lb/acre) were equally effective at controlling lettuce drop caused by *S. sclerotiorum*. A rate of 10 lb/acre is much higher that the lower rates (2-8 lbs/acre) recommended for open field and therefore the researchers cautioned that the high rate may not be an economically viable option for lettuce commercial production. In another trial in California, it was reported that best control of lettuce drop caused by *S. minor* was obtained when multiple applications of Contans were made on the same plots, after crop thinning and before disking the crop residue (K. Subbarao, University of California, Davis *pers. comm.).* In this trial, successful control was obtained only in the second and third successive lettuce crops. In the second and third crops, multiple applications of Contans applied at 2 and 4 lbs/acre significantly reduced disease incidence from about 50 % to 20 % and 60 % to 30 %, respectively, when compared to untreated plots. Both rates tested were equally effective as Endura™ in controlling the disease. Soil tests showed that Contans was also effective at reducing the sclerotia inoculum in soil when compared to the fungicide treatment and untreated control. Recently *Paenibacillus polymyxa* isolated from parasitised *S. minor* sclerotia has shown some potential for control of lettuce drop. In addition to disease suppression, *P. polymyxa* treatment also had a growth enhancing effect on lettuce and increased the lettuce head weight and yield significantly (Pryor 2007).

In Australia commercially available biological control agents including preparations of the mycoparasites *C. minitans* and *Trichoderma* spp. failed to provide control of Lettuce drop (*S. minor*) that was as effective as that provided by fungicides. It was concluded that biocontrols failed because they did not establish in soils (pre-plant treatments) and plugs of seedling transplants (nursery treatments) at levels that were required for effective biocontrol (Villalta *et al.* 2004). More recently, Villalta *et. al.* 2004 (VG01096) found that many farming practices, such as the time of application of nitrogen fertilisers and fungicide type affected the establishment of the biocontrol agent *Trichoderma atroviride* (strain C52) in soil.

Mycoparasites can be classed as aggressive or passive. For example, Adams and Ayers (1979) studied eight mycoparasites of *S. minor.* Of these only two (*Sporidesmium sclerotivorum*, recently reviewed by Fravel (2007), and *Teratosperma oligocladum*) were able to grow out of an infected sclerotium into the soil subsequently parasitising healthy sclerotia. These were termed aggressive mycoparasites. The remaining six mycoparasites including *Talaromyces flavus, Coniothyrium minitans* and *Trichoderma* sp. were classed as passive mycoparasites. As such, these remaining six are likely only to have potential in altered or noncompetitive environments (ie. in sterile, soilless or fumigated soils)(Adams 1990).

The most extensively studied mycoparasites have been the *Trichoderma* spp. and *Gliocladium virens* (Papavizas 1985). *Trichoderma* spp. have a range of reported mechanisms of activity including the production of lytic enzymes, disruption of fungal pathogenicity, induction of host plant defenses and competition for nutrients (Punja and Utkhede 2003). These fungi can be excellent mycoparasites in controlled studies (Locke *et al.* 1984; Marois and Locke 1985). In the field their efficacy is moderated by a range of biotic and abiotic factors (Vinale *et al.* 2008). *Trichoderma* spp. require at least 10^5 propagules/g soil to control a plant pathogen with an inoculum density of less than 200 propagules/g of field soil. For this reason *Trichoderma* spp. and *Gliocladium virens* are considered inefficient mycoparasites (Adams 1990).

Competition for iron

Frequently iron is scarce in the rhizosphere environment. Where iron is limiting PGPR produce low molecular weight compounds called siderophores. PGPR siderophores have a higher affinity for iron than fungal siderophores enabling PGPR to outcompete fungal plant pathogens (Loper and Henkels 1999). Suppression of Fusarium wilt of carnation *F. oxysporum* f. sp. *dianthi* and radish *F. oxysporum* f. sp. *raphani* by *Pseudomonas putida* strain WCS358 results from such a mechanism (Duijff *et al.* 1993; Lemanceau *et al.* 1992; Raaijmakers *et al.* 1995). A range of environmental factors including pH, the amount and form of iron and the presence of other elements in the soil effect the efficiency of siderophore production and therefore the effectiveness of competition for iron as a means of biological control (Duffy and Défago 1999).

Antibiosis

The study of antibiotic minus mutant strains has confirmed a role for antibiotics production in the suppression of plant disease by biocontrol agents. A number of antibiotic substances have been identified; those produced by *Pseudomonas* spp. are well characterized. They include the phloroglucinols, phenazine derivatives, pyoluteorin, pyrrolnitrin, cyclic lipopeptides and hydrogen cyanamide (Haas and Keel 2003). The antibiotics gliotoxin and gliovirin are produced by *Trichoderma virens* (Howell 2003) and the glycolipid flocculosin is an important determinant of biological control by *Pseudozyma* (Cheng *et al.* 2003). Strains of *Bacillus subtilis* are active against a wide range of soilborne pathogens including *Fusarium* spp., *Pythium* spp., *Rhizoctonia* spp. and *Sclerotinia minor* (Copping 2004). The inhibitory effects of this biological control agent are due in part to its production of an antifungal antibiotic (Chitarra *et al.* 2003).

Degradation of virulence factors

Many PGPR are able to detoxify pathogen toxins or degrade pathogen cellular signals thereby disrupting the expression of virulence genes (Compant *et al.* 2005). Of particular relevance to the current study is the ability of some BCA or PGPR to degrade oxalate or hydrolyse fusaric acid. Several microorganisms including *Burkholderia cepacia* and *Ralstonia solanacearum* can hydrolyse fusaric acid, a phytotoxin produced by *Fusarium* species (Toyoda *et al.* 1988; Toyoda and Utsumi 1991). The pathogenic ability of *S. sclerotiorum* depends upon its ability to produce oxalic acid. Degradation of this oxalic acid by oxalate-degrading bacteria has been shown to protect *Arabidopsis thaliana* against *S. sclerotiorum* infections (Schoonbeek *et al,* 2007).

Enzyme production

The ability of many PGPR to antagonise or parasitise plant pathogenic fungi depends upon their production of enzymes. For example, the antagonistic ability of *Serratia marcescens* against *Sclerotium rolfsii* (Ordentlich *et al.* 1988) and *Paenibacillus* sp. strain 300 and *Streptomyces* sp. strain 385 against *Fusarium oxysporum* f. sp. *cucumerinum* (Singh *et al.* 1999) is due to their production of extracellular chitinases. Chitinolytic activity has also been reported for an endophytic strain of *Bacillus cereus* (Pleban *et al,* 1997). Synthesis of β-1,3-glucanase by *B. cepacia* destroys cell walls of *R. solani, S. rolfsii* and *P. ultimum* (Fridlender *et al.* 1993). Both chitinase and β-1,3-glucanase are responsible for cell wall degradation in *R. solani* and *S. rolfsii* by *T. harzianum* (Elad *et al.* 1982). These two enzymes are also involved in the inhibition of *R. solani* by fluorescent *Pseudomonas* GRC_3 (Arora *et al.* 2007). Proteases are involved in the biological control of *S. sclerotiorum* by *Serratia plymuthica* (Kamensky *et al.* 2003).

Hypovirulence and avirulence

Elliston (1982) defines hypovirulence in fungal plant pathogens as the reduced ability of selected isolates within a population of a pathogen to infect, colonize, kill and (or) reproduce on susceptible host

tissues. Historically the term refers to reduced virulence brought about by infection of the pathogenic fungus by a virus or virus like agent. The classic example of hypovirulence comes from chestnut plantations in Italy, Belgium and France where, during the early 1970s chestnut blight cankers were observed to spontaneously heal. Hypovirulent isolates of *Cryphonectria parasitica* (the chestnut blight fungus) contained a single cytoplasmic element of double-stranded RNA (dsRNA) a result of infection of the fungus by fungal viruses in the family Hypoviridae (Milgroom and Cortesi 2004). This could be transmitted through the virulent population by anastomosis of compatible strains. Whilst hypovirulence is often caused by infection of fungal viruses and associated dsRNA elements, these are not predictive indicators of the condition. In a study of isolates of *F. oxysporum* for example, some hypovirulent isolates contained dsRNA, while other hypovirulent isolates did not. Further, of six dsRNA-containing isolates, three were hypovirulent and three were not (Kilic and Griffin 1998). A similar observation has been made for *S. sclerotiorum* (Boland 1992; Zhou and Boland 1998a), *S. minor* (Melzer and Boland 1996) and *S. homoeocarpa* (Zhou and Boland 1997). Several other factors including plasmids, mitochondrial and nuclear mutations are also potentially associated with hypovirulence (Monteiro-Vitorello *et al.* 1995; Nuss and Koltin 1990).

In the wider literature the terms non-pathogenic and hypovirulent are often used interchangeably to describe fungal isolates that produce little or no disease. Strictly speaking, non-pathogenic strains are avirulent, incapable of causing disease and the mechanisms of control are quite different. The classification of strains as either transmissible hypovirulent strains (through transmission of dsRNA mycoviruses) or non transmissible, non-pathogenic or hypovirulent strains by (Sneh 1998) is helpful in this respect.

Transmissible hypovirulent strains

Transmissible hypovirulent strains of *F. oxysporum* (Kilic and Griffin 1998), *S. sclerotiorum, S. minor* and *S. homoeocarpa* (Boland 2004) have been identified. Several mechanisms of activity have been identified for these transmissible hypovirulent strains including reduced or delayed production of oxalic acid in *S. sclerotiorum* (Zhou and Boland 1999), mitochondrial disfunction in *S. homoecarpa* (Boland *et al.* 2000; Deng *et al.* 2003) and production of a chemical, 1-hydroxy-2,6-pyrazinedione (sclerominol), of unknown function in *S. minor* (Savard *et al.* 2003).

There have been relatively few field based studies of the biocontrol potential of transmissible hypovirulent strains. The best example of field scale use of this method of biocontrol is the chestnut blight fungus which has been studied for over thirty years. Milgroom and Cortesi (2004) report that hypovirulence has controlled chestnut blight well in some locations in Europe and in Michigan, USA. In contrast, biological control has largely failed in eastern North America. Success in the biological control of this pathogen using hypovirulence depends upon the virus, fungus, tree and environment and still more work is needed to understand the factors regulating the establishment of hypovirulence (Milgroom and Cortesi 2004). The potential for using hypovirulent isolates as biological controls depends, at least in part, on the ability to transfer hypovirulence from hypovirulent isolates to virulent isolates. For *Sclerotinia* spp. three issues are of relevance (Boland 2004). Firstly mycoviruses have no known vector for transmission between isolates. Secondly cytoplasmic (Hypoviruses) and mitochondrial (Mitoviruses) viruses known to be associated with hypovirulence in *Sclerotinia* spp. are not transmitted by ascospores. Finally, since transmission occurs through hyphal anastomosis, it is restricted to vegetative compatibility groups (VCGs). Field application of hypovirulence as a means of biological control of *Sclerotinia* spp. has therefore focused on *S. minor* and *S. homeocarpa* since *S. sclerotiorum* is known to have many vegetative compatibility groups (Boland 2004).

Spraying hypovirulent isolates of *S. minor* onto established lesions of compatible VCGs reduced lesion size by up to 50 % and the number of sclerotia on diseased leaves by more than 90 % (Melzer and Boland 1996). Likewise, the hypovirulent isolate of *S. homoeocarpa* Sh12B suppressed dollar spot on creeping bent grass by up to 58 and 80 % in naturally occurring and artificially inoculated trials respectively (Zhou and Boland 1998a; Zhou and Boland 1998b). In the field with naturally occurring disease the level of control achieved was equivalent to treatment with chlorothalonil (Zhou and Boland 1998b).

Non transmissible, non-pathogenic or hypovirulent strains

Non transmissible hypovirulent strains are often referred to as non-pathogenic strains in literature concerning *Fusarium, Pythium* and *Rhizoctonia* species. These strains are often a significant biological component of disease suppressive soils. The modes of protection by these strains are the same as some of those described elsewhere in this review for PGPR, BCA and endophytes. They include competition for infection sites and nutrients and induction of systemic resistance in the host.

For example, colonization of roots by non-pathogenic *Fusarium* spp. has been shown to suppress Fusarium wilt because root colonization by the non-pathogenic strains acted as a microbial barrier to prevent contact between the pathogen and the root surface (Alabouvette *et al.* 1984). In addition non-pathogenic strains of *Fusarium oxysporum* are known to induce systemic resistance against Fusarium wilt (Larkin and Fravel 1999; Larkin *et al.* 1996) and to compete with pathogenic strains for carbon (Lemanceau *et al.* 1992; Lemanceau *et al.* 1993). These non-pathogenic strains differ in their efficacy, mode of action and dose required to suppress disease (Larkin and Fravel 1998; Larkin and Fravel 1999).

Certain non-pathogenic *Rhizoctonia* isolates can provide significant protection against pathogenic *Rhizoctonia, Pythium* (Sneh and Ichielevich-Auster 1998), and *Fusarium oxysporum* f.sp. *radicis lycopersici* (Louter and Edgington 1990). Sneh and Ichielevich-Auster (1998) found that 33 % (14) of their non pathogenic *Rhizoctonia* isolates protected more than 60 % of cabbage seedling against *R. solani.* The best eight of these isolates protected 73-95 % of cucumber seedlings from damping off due to *R. solani.* In this case, protection was obtained in seven separate pot experiments (3 for *R. solani,* 2 for *Pythium aphanidermatum* and 2 for *Pseudomonas syringae* pv. *Lachrymans*). Likewise Sneh *et al.* (2004) report that 13 of 92 hypovirulent *Rhizoctonia* isolates from New Zealand field soils provided greater than 50 % protection to radish seedlings against damping-off caused by *R. solani* in a screening experiment. However, this number was reduced to three in a subsequent experiment.

Non pathogenic *Rhizoctonia* spp. isolates have been reported for *R. solani* AGs 1,2,3,4, 5 and 10, *R. zeae,* and binucleate *Rhizoctonia* spp. AGs A, Ba, Bb, B(o), F, H, J, K, L, M, N, P, R, and S (reviewed by Sneh 1998). Since non pathogenic *Rhizoctonia* isolates are often observed to form dense mycelial mats around plant surfaces they may compete with pathogenic isolates. However, in a review on the subject, Sneh (1998) concludes that there is insufficient experimental data to support competition for nutrients as a possible mechanism of protection. Physical competition to occupy infection sites is possible (Sneh *et al.* 1989). Non pathogenic *Rhizoctonia* spp. are best known for their ability to induce systemic resistance (Cardoso and Echandi 1987; Sneh and Ichielevich-Auster 1998). In one instance it is proposed that this resistance was mediated by the activity of pectolytic enzymes of a non pathogenic binucleate *Rhizoctonia* isolate (Villajuan-Abgona *et al.* 1996).

A number of non pathogenic *Pythium* species including *P. oligandrum, P. achanticum, P. nunn, P. mycoparasiticum, P. periplocum, P. monospermum* and *P. acanthophoron* are known to control a wide variety of *Pythium* spp. (Jones and Deacon 1995; Sneh 1998). These non-pathogenic *Pythium* spp. act mainly as mycoparasites. For example, *P. oligandrum* has been shown to trap and penetrate mycelium of *P. ultimum* (Tomio and Takio 1998) and degrade the cell walls of conidia of *Fusarium* (Davanlou *et al.* 1999). *P. acanthophoron* causes rapid lysis or cytoplasmic coagulation of the host following hyphal interaction (Jones and Deacon 1995). *P. nunn* coils around and subsequently lyses the hyphae of *P. ultimum* and *P. vexans* but forms appressoria-like structures and parasitises the hyphae of *P. aphanidermatum, R. solani, Phytophthora parasitica* or *P. cinnamomi* (Lifshitz *et al.* 1984). In addition to these directly parasitic effects, *Pythium* spp. also compete directly with soilborne pathogens for nutrition from root exudates and stimulate plant growth rendering them less susceptible to disease attack (Copping 2004). Many mycoparasitic species of *Pythium* have a wide host range. For example, *Pythium oligandrum* parasitises a range of soilborne pathogens including *Rhizoctonia solani* and a number of pathogenic species of *Pythium* and *Fusarium* (reviewed by Brožová 2002). Commercial formulations of this biocontrol agent, manufactured by fermentation are sold as 'Polyversum' (Remeslo) and 'Polygandron' (Biopreparaty) (Copping 2004).

Commercial use of PGPR, BCA and endophytes

In spite of the enormous amount of literature that is available on biological control agents there are relatively few examples of their successful commercial use, particularly in the vegetable industry. The overwhelming majority of biological products available commercially consist of a high concentration of only one active microbial strain. Sturz (2006) points out the basic flaws in such an approach. Firstly, extracellular signalling molecules accumulate within large population densities of individual bacterial strains. These can modulate a range of metabolic processes, some of which are incompatible with the goals of biological control. In addition, application of massive quantities of a single bacterial species will alter the physiology and niche behaviour of the BCA and may elicit a generalised antagonistic response from the resident population. This author argues the value of traditional (often timeconsuming) approaches such as conservation tillage, 'creative fallow' options, manuring, long term crop rotation and compatible cropping systems as a means of modifying the soil microbial agroecosystem at the expense of pathogen populations and encouraging disease suppressiveness.

Key technical issues that limit the commercial use of biological control agents are reviewed in detail by Butt and Copping (2000). Issues of immediate relevance to the vegetable industry include the:

1. cost of the biological control agent (Often crop management strategies that include a biological control agent are at least as expensive as the chemical only option).
2. ecological fitness of the biological control agent (The soil environment is complex and biological control agents must not only out compete other soil microorganisms but must be able to tolerate a wide range of climatic and soil conditions. This is the primary reason for the failure of an otherwise effective biological control agent in the field.)
3. speed of activity (Often biological control agents act slowly or take time to establish in the soil environment. In intensive vegetable production systems the rapid turn over of crops can leave little time for establishment of a biological control agent).
4. method and timing of application (to target the pathogen).

As the study of biological control progresses from laboratory plate tests and greenhouse pot-based assays to commercial application, the importance of understanding the epidemiology of the pathogen and the ecology of the pathogen and chosen biological control agent in the soil environment is becoming clear. The example provided below demonstrates how an improved understanding of epidemiology and ecology can address each of the four issues above and make biological control a commercial proposition for the management of soilborne pathogens in vegetable production.

PGPR, BCA and endophytes are generally considered preventative treatments therefore early application is essential. Direct application to soil is often impractical. For example, the potential of *Coniothyrium minitans* as a biological control agent against *Sclerotinia* diseases has been demonstrated by numerous research groups (Huang 1980; Tribe 1957). However, in one study (Huang 1980) the amount of *C. minitans* product used (1 kg per 6 m of seed furrow) would be completely impractical in commercial practice. Likewise, Adams (1990) estimates that, using traditional methods of application, approximately 110-220 kg/ha (100 spores/g of field soil) of *Sporidesmium sclerotivorum,* an aggressive mycoparasite of *Sclerotinia* spp. would be required for adequate disease control and that this would cost the farmer between $1100 and $3300/ha. After extensively researching the epidemiology of lettuce drop and the ecology of the pathogen and mycoparasite in soil, Adams (1990) further proposed that if a low dose of this mycoparasite could be applied to the diseased lettuce crop immediately after harvest, before disking, the likelihood of macroconidia of the parasite being next to sclerotia of *S. minor* in the soil profile would be greatly increased. These sclerotia would rapidly become infected and mycelium of the parasite growing out from infected sclerotia would rapidly infect all sclerotia within a cluster. This author subsequently tested his hypothesis obtaining 53 % disease control in the third lettuce crop following application of 0.2 kg/ha *S. sclerotivorum* (Adams and Fravel 1990). By the end of the fifth season *S. sclerotivorum* had eradicated or nearly eradicated the pathogen from all plots, including the control plots (Fravel 2007). The estimated cost of treatment at this rate of application is US $2 (Adams and Fravel 1990). *S. sclerotivorum* is a biotroph and is difficult to culture. It is slow to act and is unlikely to provide control in the season in which it is applied. However, it is compatible with several fungicides, has no known non-target effects and multi-season control can be achieved with a single application of 0.2 kg/ha (Fravel 2007).

This method of applying mycoparasite preparations to a diseased crop has been proposed by the same author as a means of improving the level of control currently achieved using several other mycoparasites. The fungal parasites *P. nunn* and *Trichoderma* spp. do not produce new resting structures as a result of parasitism. New propagules are formed as a result of saprophytic growth on fresh organic matter. Adams (1990) therefore suggests that application of the mycoparasite to crop residue after harvest, before the residue is incorporated into the soil would enable the mycoparasite to increase its population on the organic matter whilst also parasitising any propagules produced on the crop.

Also, since *C. minitans* is not generally considered to be an aggressive mycoparasite in soil (Adams 1989) but infects sclerotia of *Sclerotinia* spp. readily on host tissue (Huang 1977), this mycoparasite may also be more effective if applied to the diseased crop following harvest (Adams 1990).

The addition of biological control agents to the cropping system is not a simple process since the application must be cost effective and timed to optimise the potential for the BCA to prevent disease. For example, early application of *C. minitans* (ie. six weeks prior to planting) results in a decline in colony numbers during the preplanting period. Application at planting is currently more effective (Ridgway *et al.* 2001) although treatment of the diseased crop following harvest has also been proposed as a means of reducing the build up of inoculum (Adams 1990).

Non-pathogenic *Pythium* formulations have most frequently been evaluated as seed treatments. Treatment of tomato seeds with zoospores of *P. oligandrum* has been shown to reduce seed rot and damping-off caused by *P. ultimum* and *Rhizoctonia solani* by 79 % and 64 % respectively (He *et al.* 1992). Seed treatment with oospores of *P. oligandrum* was as effective as chemical seed treatment at controlling pre-emergence damping-off caused by *P. ultimum* in sugar beet (Martin and Hancock 1987).

A number of commercial preparations of *Trichoderma* spp. are available. Commercialisation of *T. virens*, known previously as *Gliocladium virens*, is reviewed by Lumsden and Knauss (2007). The commercial product GlioGard™ an alginate-wheat-bran prill was reformulated due to an increase in the cost of the alginate and difficulties drying large quantities of the alginate prill. It is currently sold as dry granular product, SoilGuard™. In this form it has been effectively used to control damping-off diseases in transplant production and horticultural greenhouse production systems but is currently not cost effective for large-scale vegetable production (Lumsden and Knauss 2007). Development of an improved formulation that can be mixed with water for field application as a drench or through drip application is currently in progress (Lumsden and Knauss 2007).

Regardless of the rate, method or timing of application, the survival of the BCA in the chosen formulation is paramount. Being living organisms BCAs have a limited shelf life even under optimal conditions. A range of factors can reduce the shelf life of BCAs. Powdered formulations often have a longer shelf life than liquid preparations. A powdered formulation of a *P. oligandrum* biological control agent remained viable and active for at least three years whereas liquid preparation lost its activity quickly (Vesely and Hejdnek 1984). Temperature affects the survival of *Pseudomonas fluorescens* applied to seed using a patented biopolymer technology. High cell numbers were maintained on seed stored at 4°C for up to 70 days but at 20°C bacterial numbers declined (O'Callaghan *et al* 2006). Even factors such as the type of packaging of treated seeds has been shown to affect BCA survival (O'Callaghan *et al* 2006).

1.12.3 Mycorrhizal fungi

Through their symbiotic relationship with the host plant mycorrhizal fungi can protect plant roots from soilborne diseases using many of the same mechanisms described for PGPR, BCAs and endophytes. There are seven known types of mycorrhiza. Of these the ectomycorrhiza and vesicular arbuscular mycorrhiza (VAM) are the most important in agriculture (Zeng 2006). These fungi protect plants from root diseases by (i) providing a physical barrier to infection, (ii) competing with the pathogen, (iii) producing allelopathic chemicals (eg. secondary metabolites, and antagonistic chemicals such as antibiotics and toxins or altering the amount and type of root exudate), (iv) eliciting an induced systemic resistance response in the host (Xavier and Boyetchko 2002; Zeng 2006).

The potential of mycorrhizae for the biological control of crown and root rot of tomato caused by *F. oxysporum* f. sp. *lycopersici* has been demonstrated (Caron *et al.* 1986). Reduction in wilting caused by the pathogen was attributed, in part to increased lignin deposition in the host plant cell walls that may have restricted the pathogen (Dehne and Schönbeck 1979). The mycorrhizal fungus *Glomus fasciculatum* applied together with *T. harzianum* has been shown to control damping-off caused by *P. aphanidermatum* in tobacco seedlings (Sreeramulu *et al.* 1998). A range of mycorrhizal species are effective against *S. rolfsii* on ground nut (Kulkarni *et al.* 1997).

Mycorrhizal fungi are not well utilised in agriculture generally but specifically in vegetable cropping systems. This is due in part to the obligately biotrophic status of most species and the lack of appropriate technology for mass production (Xavier and Boyetchko 2002). Other specific factors restrict their use in vegetable production. Some vegetable crop species, for example the brassicas, simply do not form mycorrhizal associations. In addition, vegetable cropping soils often have higher than optimal amounts of N and P fertilisers. This can severely impair the nodulation and mycorrhizal symbioses (Xavier and Boyetchko 2002).

1.13 Self defence

A complex and coordinated resistance response is activated in plants upon recognition of a pathogen. This leads to the induction of a range of host responses including phytoalexin synthesis, physical barriers such as hypersensitive cell death, callose and lignin deposition, and the production of chemicals such as proteinase inhibitors and lytic enzymes (eg. glucanases and chitinases). When the

plant fails to recognise the pathogen or the pathogen is able to avoid or overcome the plant resistance response, disease develops.

Historically this ability of the plant to defend itself has often been overlooked. Increasingly however, crop protection experts are looking within the plant as they seek to accelerate and exploit the plant resistance response and search for novel chemistries. Understanding the mechanisms of plant defence against pathogen invasion is critical as these dictate which chemistries, are likely to accelerate or amplify an effective host response against which pathogens. Broadly pathogens can be classified as biotrophs, those that feed on living host tissue, and necrotrophs, those that kill host tissue and feed on its remains. Hemibiotrophy defined by Perfect and Green (2001) as an initial period of biotrophy followed by necrotrophic hyphae is a subsidiary definition. Considering pathogens as either biotrophic or nectrotophic, it is easy to understand how plants might respond to their invasion differently. A hypersensitive response with programmed cell death for example might be an effective strategy against a biotrophic pathogen but may well support the growth of a necrotroph by supplying an instant food source.

Many of the soilborne plant pathogens are predominantly necrotrophic. *Sclerotinia* spp. are classic necrotrophs. These ascomycete fungi kill tissue as they spread. Pathogenicity results from the production of oxalic acid which is toxic to most plants and polygalacturonase which breaks down plant cell walls (Wang and Fristensky 2001). Likewise the basidiomycete fungus *Rhizoctonia solani* is also considered to be a necrotropic pathogen. Wilt fungi including the ascomycete fungi of the genus *Fusarium* are generally considered to be necrotrophic. However, these fungi initially live biotrophically before reaching and colonizing the xylem vessels. Invasion of other tissues occurs only after the xylem vessels have become blocked by fungal and degraded cell wall material causing wilting and cell death. They may therefore be considered closer to biotrophs than necrotrophs (Thaler *et al.* 2004). *Pythium* spp. are oomycetes and as such are taxonomically distinct from fungi, although they use infection strategies that have much in common (Latijnhouwers *et al.* 2003). These pathogens are classified in the kingdom *Protoctista* and members of this genus exhibit necrotrophic or hemibiotrophic lifestyles.

The molecular mechanisms underlying the defence responses of biotrophic and necrotrophic pathogens have been extensively reviewed by a number of research groups, for example, Hammond-Kosack and Jones (1997), Oliver and Ipcho (2004) and Glazebrook (2005). According to Glazebrook (2005), with respect to several subtleties, in general, gene-for-gene resistance and salicylic acid dependent signalling are effective against biotrophs whereas jasmonate and/or ethelyene signalling is effective against necrotrophic pathogens of *Arabidopsis thaliana.* Oliver and Ipcho (2004) also report the ability of this 'mode-of-defence division' to distinguish necrotroph from biotroph but caution that this limits the biotroph class to those pathogens that possess haustoria.

Most of the work in this field has utilised *Arabidopsis thaliana* and the suite of pathogens to which it is susceptible as a model plant-pathogen system (Glazebrook 2005; Oliver and Ipcho 2004; Thomma *et al.* 2001; Thomma *et al.* 1998; Vijayan *et al.* 1998). However, recent work conducted using *Lycopersicon esculentum* (tomato) reports that the jasmonate response is involved in limiting susceptibility to pathogens from a wide range of taxonomic groupings and lifestyles thus challenging the generalised biotrophic and necrotrophic *Arabidopsis* models of plant defence (Thaler *et al.* 2004). Perhaps this may be because true biotrophs and true necrotrophs are relatively rare with far more pathogens, described by Parbery (1996) as 'predominantly biotrophic hemibiotrophs' or 'predominantly necrotrophic hemibiotrophs', falling somewhere along the continuum between biotroph and necrotroph. Further, not all species within a particular genus will group together on this continuum.

Whilst it is not possible to generalize plant defence responses based on the mode of nutrition of the pathogen or any other predictive indicator, we do know that plants are equipped with a host of defence mechanisms. These are activated by salicylic acid, jasmonate and/or ethylene dependant signalling pathways (Fig. 1.2). Understanding which pathway is most important for which pathogen and how the pathogen regulates these pathways will lead to improved crop protection practices as the ability to accelerate or upregulate plant defence responses improves.

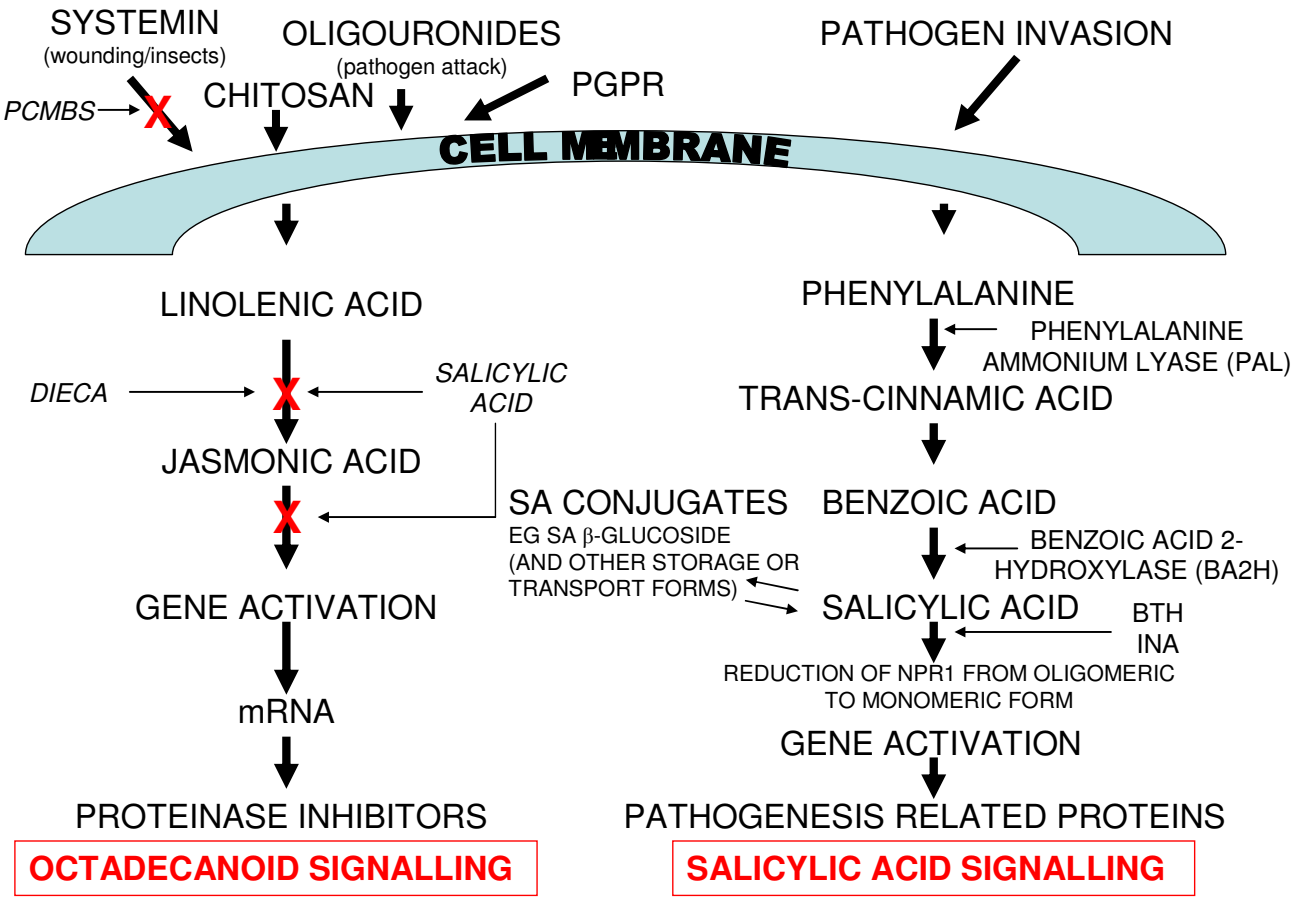


Figure 1.2 Plant defence responses mediated by jasmonic acid via the octadecanoid signalling pathway or by Salicylic acid. Inhibitors (sodium *p*-chloromurcuribenzenesulfonate, PCMBS; sodium diethyldithiocarbamate, DIECA; salicylic acid) shown in italics. (Based on Doares *et al.* 1995; Mauch-Mani and Métraux 1998; Ryals *et al.* 1996).

1.14 Induced resistance

Induced resistance is a state of heightened defensive capacity in plant species. It can be mediated by biotic and abiotic elicitors (Lyon *et al.* 1995) and results in long-lasting protection against a broad spectrum of microorganisms including viruses, bacteria, fungi and oomycetes (Ryals *et al.* 1996; Sticher *et al.* 1997). The two most clearly defined forms of induced resistance are systemic acquired resistance (SAR) and induced systemic resistance (ISR), which can be differentiated on the basis of the nature of the elicitor and the regulatory pathways involved (Vallad and Goodman 2004).

SAR can be triggered by microbial infection (virulent, avirulent or non-pathogenic microbes) or with chemicals. It results in the accumulation of salicylic acid (SA) and pathogenesis-related proteins and is effective across a wide variety of plant species. By contrast ISR is triggered by plant growth-promoting rhizobacteria (PGPR) (eg. *Pseudomonas* spp, (Zhou and Paulitz 1994)), is mediated by jasmonate and ethylene and the ability of certain PGPR to elicit ISR is specific for certain plant species

and genotypes. Common to both of these mechanisms is the lack of any direct antimicrobial effect therefore they offer a means of controlling plant disease without asserting direct selective pressure on pathogen population (Vallad and Goodman 2004). In addition, the biological and synthetic elicitors compare very favourably with current pesticides in terms of their environmental impact. For these reasons induced resistance, including SAR and ISR is very compatible with sustainable integrated pest management based approaches to plant disease control.

1.14.1 Systemic acquired resistance (SAR)

For a century scientists have observed that plants that are able to survive pathogen infection sometimes develop increased resistance to subsequent infections. This phenomenon was termed physiological acquired immunity by Chester (1933). In 1961 A. Frank Ross demonstrated that tobacco mosaic virus infections were restricted by prior infection. This resistance was also effective against tobacco necrosis virus and several bacterial pathogens (Ross 1961) and is widely accepted as the first systematic study of SAR. In the 1980s a number of SAR models were developed. In addition to tobacco (*Nicotiana tabacum* L.) SAR has been extensively studied in cucumber (*Cucumis sativus* L.), common bean (*Phaseolus vulgaris* L.), rice (*Oryza sativa* L.) and *Arabidopsis thaliana* (L.) Heynh and found to be effective against a broad range of viral, bacterial and fungal pathogens (Vallad and Goodman 2004). The science in this field is developing rapidly and has been the subject of several reviews (eg. Ryals *et al.* 1994; Ryals *et al.* 1996; Sticher *et al.* 1997). In the last 20 years molecular and biochemical studies have revealed much about the mechanisms underpinning the SAR response (reviewed by Durrant and Dong 2004). This work has led to the development of synthetic inducers, several of which have recently been made available commercially. The effectiveness of these inducers, their ability to induce a SAR response capable of controlling plant disease in the field, is only now being established.

1.14.2 Natural elicitors

Salicylic acid

The phenolic molecule salicylic acid (SA) is synthesised by plants in response to challenge by a diverse range of pathogens and is an essential signalling molecule regulating changes in gene expression. Salicylic acid is produced in response to pathogen invasion by conversion of phenylalanine to *trans*-cinnamic acid, catalysed by phenylalanine lyase. *Trans*-cinnamic acid is converted to benzoic acid. Hydroxylation of this molecule, catalysed by benzoic acid 2-hydroxylase results in the production of salicylic acid (Ryals *et al.* 1996) (Fig. 1.2). Most of the pathogen induced SA is glucosylated to form SA β-glucoside that is sequestered in vacuoles and serves as a readily hydrolysable source of SA (Loake and Grant 2007). Other modified forms such as methylated SA and amino acid conjugated SA have been implicated as transport forms of SA (Loake and Grant 2007).

SAR requires changes in gene expression in plants (Fig. 1.2). Accumulation of SA changes cellular redox potential (Fobert and Després 2005). This triggers reduction of NON-EXPRESSOR of PATHOGENESIS RELATED1 (NPR1) protein from disulphide-bound oligomers to active monomers (Mou *et al.* 2003). These translocate to the nucleus where they interact with TGA transcription factors. These TGA factors bind to SA-responsive elements in the promoters of PATHOGENESIS RELATED (PR) genes resulting in SAR (Grant and Lamb 2006). The molecular basis of SA synthesis and regulation of the SA-dependant signalling pathway leading to defense gene expression is reviewed in detail by Durrant and Dong (2004) and Katagiri (2004).

Field use of SAR to control crop diseases has, until recently, centred on the use of SA and a derivative, acetylsalicylic acid (aspirin). Salicylic acid and acetylsalicylic acid as a seed dressing or soil drench have been shown to significantly reduce the percentage root rot incidence at both pre- and post- emergence stages of lupin plant growth in soils artificially infested with *Fusarium solani, Rhizoctonia solani* and *Sclerotium rolfsii* (El-Mougy 2004). In addition to any SAR effects, a direct inhibitory effect of SA and acetyl SA on the growth of these pathogens has been demonstrated (El-Mougy 2002). A significant reduction in *Fusarium* head blight in wheat has been reported following treatment with a sodium salt of SA (Zhang *et al.* 2007). In kiwifruit vines SA caused a 35 % reduction in disease caused by *Sclerotinia sclerotiorum* (Reglinski *et al.* 2001). Elsewhere exogenous application of SA failed to induce systemic resistance to cucumber root rot caused by *Pythium aphanidermatum* (Chen *et al.* 1999).

SA is available in Australia as ReZist® (Stoller) and has been used effectively to induce SAR in melon trials (McConchie 2007).

Jasmonic acid

Jasmonic acid and its methyl ester are naturally occurring plant growth regulators. Jasmonic acid is synthesised from linolenic acid in plant cells via the octadecanoid pathway (Fig. 1.2). It has diverse roles in plant development including senescence, leaf abscission and inhibition of germination (Creelman and Mullet 1995). In addition jasmonates induce a number of genes that are involved in plant defence including enzymes involved in flavonoid (chalcone synthase and phenylalanine ammonia lyase) (Creelman *et al.* 1992; Gundlach *et al.* 1992) and sesquiterpenoid biosynthesis (hydroxymethylglutaryl Co A reductase)(Choi *et al.* 1994) antifungal proteins thionin (Andresen *et al.* 1992) and osmotin (Xu *et al.* 1994) and proteinase inhibitors (Farmer and Ryan 1990).

Application of jasmonic acid or its methyl ester induced local and systemic protection of potato and tomato plants against *Phytophthora infestans* (Cohen *et al.* 1993). However, at high concentrations symptoms of phytotoxicity were observed. This fact together with the wide range of physiological effects of the jasmonates make it seem unlikely that this group will ever form the basis of a practical disease control strategy (Lyon *et al.* 1995).

Complex carbohydrates (eg. Chitin, chitosan & glucans)

The most widely studied complex carbohydrate elicitor is chitin and the chemically related polymer chitosan. Chitin is the principal cell wall material in most fungi. It also occurs naturally in arthropods with shrimp and crab processing wastes being the primary source of the compound for agricultural purposes. Chitin is a polymer of *N*-acetyl-D-glucosamine residues linked by β-1-4 bonds. Chitosan, 2-amino-2-deoxy-beta-D-glucosamine, is the deacetylated derivative of chitin following treatment with strong alkalis (Peniston and Johnson 1980).

In contrast to other elicitors, chitosan is an antifungal compound directly inhibiting the growth of several fungi including *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Benhamou *et al.* 1998), f. sp. *albedinis* (El Hassni *et al.* 2004), *Pythium aphanidermatum* (El Ghaouth *et al.* 1994), *Rhizoctonia solani, Fusarium solani* and *Sclerotium rolfsii* (Abd-El-Kareem *et al.* 2006). Soil application of chitin and chitosan has been shown to increase root chitinase activity (Abd-El-Kareem *et al.* 2006). Chitinases are able to hydrolyse chitin, a major component of fungal cell walls. In addition, chitin amendments can alter soil microflora resulting in an increase in soil microorganisms with the ability to break down chitin (Godoy *et al.* 1983). Decomposition of chitin in soils also releases volatiles (eg. ammonia) which suppress some soilborne pathogens including *Rhizoctonia* (Sneh and Henis 1972).

As an elicitor chitosan causes linolenic acid to be released from the plant cell membrane, possibly via activation of a lipase (Doares *et al.* 1995). Linolenic acid is converted to jasmonic acid which causes gene activation leading to the expression of defence genes including proteinase inhibitors and polyphenol oxidase (Doares *et al.* 1995). This defence pathway is known as the jasmonic acid-dependant or octadecanoid signalling pathway (Fig. 1.2).

Soil application of chitin or chitosan has been effective against a range of soilborne pathogens, although it is not clear in most of the reports which of the above mechanisms accounted for the observed reduction of disease. Under greenhouse conditions soil application of chitin or chitosan reduced the incidence of tomato root rot by at least 88.0, 86.2 and 83.1 % in soil infested with *Rhizoctonia solani, Fusarium solani* and *Sclerotium rolfsii* respectively (Abd-El-Kareem *et al.* 2006). In the field, soil applied chitin and chitosan applied as a root dip had no effect on soil populations of *Fusarium oxysporum.* The root dip treatment significantly reduced disease only in trials conducted using a tolerant celery cultivar. Soil application significantly reduced incidence and severity of Fusarium yellows in the susceptible and tolerant celery crops (Bell *et al.* 1998). In this work both treatments were applied immediately before planting. Given some of the mechanisms described above, a longer time between treatment application and planting may have further improved the efficacy in these trials.

In Australia a large study was conducted for the Rural Industries Research and Development Corporation to evaluate the potential for chitosan to enhance plant defence (Walker *et al.* 2004). Trials were conducted using tomatoes, carrots, cucumbers, peas, snow peas, capsicums, beetroot and lettuce. Phytotoxicity was not observed in any of these crops and the authors conclude that trial results were most promising in tomatoes. The majority of the trials reported in this work used Aminogro® (Organic Crop Protectants) as the sole source of chitosan as it was reportedly the only commercial source of chitosan available in Australia. This is very problematic since the suppliers market the product as an amino acid/nutritional supplement and make no claims regarding its chitosan content. In the few trials where Aminogro® is compared with a Chinese chitosan product, reported plant growth, yield and disease effects are confounded by the nutritional supplements in the Aminogro® product.

A final potential use for chitin is as a medium for the production of biological control agents. In this respect *Bacillus cereus* QQ308 produces antifungal hydrolytic enzymes (eg. chitinase, chitosanase

and protease) when grown in a medium containing shrimp and crab shell powder (Chang *et al.* 2007). Supernatant from the *B. cereus* culture inhibited the growth of *Fusarium oxysporum, F. solani* and *Pythium ultimum in vitro.*

Ethylene

Ethylene is a plant hormone with well established regulatory effects including ripening for which it is well known, also seed germination, regulation of flowering, abscission of various organs and senescence (Chang and Meyerowitz 1995). Ethylene elicits a range of host responses. These have been reviewed briefly by Lyon *et al.* (1995) and include induction of chitinase, glucanases or pathogenesis related proteins in specific crops. Ethylene, as Ethrel®, has been used to increase the resistance of rice plants to rice blast disease (Sekizawa and Mase 1981). Ethylene has not been widely used to induce a resistance response in the field. This is probably because it is not specific and it also induces other less desirable changes in plant physiology (Lyon *et al.* 1995).

1.14.3 Synthetic elicitors

An increasing number of synthetic chemicals have been tested for their ability to elicit host plant defences against pathogen invasion. Of these, two compounds, both functional analogues of salicylic acid, have been intensively studied, 2,6-dichloroisonicotinic acid (INA) and benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH) also known as acibenzolar-S-methyl or benzothiadiazole. Use of these inducers has been reviewed by Vallad and Goodman (2004). This work reviews 37 field studies using a range of host-pathogen pairs. With the exception of only two studies (both increased the severity of late leaf spot caused by *Cercosporidium personatum* in peanut), application of either INA or BTH reduced disease relative to the untreated control by between 4 and 99 %.

SAR responses elicited by INA or BTH have been demonstrated to be effective against a number of soilborne pathogens for a range of host crops. White mold (caused by *Sclerotinia sclerotiorum*) of soybean was reduced by 46 and 59 % using INA and BTH respectively to elicit SAR (Dann *et al.* 1998). A significant reduction in Fusarium head blight in wheat has also been reported following treatment with INA or β-amino-*n*-butyric acid (BABA), another functional analogue of SA (Zhang *et al.* 2007). In tomato plants BTH protects plants against disease caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* by restricting the pathogen to the epidermis and outer cortex through the formation of callose-enriched wall appositions at sites of fungal invasion (Benhamou and Bélanger 1998).

Phytotoxicity issues have hindered the commercial development of INA. However, BTH has been commercialized as Bion® (Syngenta). This product is available in Australia and it is currently marketed as a seed treatment to the cotton industry. Whilst one litre of produce treats huge quantities of seed, at A$67,000/L this product is far from being within reach of the vegetable industry.

Tiadinil (*N*-(3-chloro-4-methylphenyl)-4-methyl-1,2,3-thiadiazole-5-carboxamide) was recently developed as a novel class of agent to control rice blast disease. Like BTH, tiadinil contains a thiadiazole skeleton. This compound is marketed in Japan as V-GET® (Nihon Nohyaku Co., Ltd.) for the control of rice blast. In trial work it has been shown to induce resistance against *Pseudomonas syringae* pv. *tabaci* (Yasuda *et al.* 2004). In subsequent work SAR was induced against the same pathogen by a metabolite of tiadinil (SV-03, 4-methyl-1,2,3-thiadiazole-5-carboxylic acid) (Yasuda *et al.* 2006).

Other chemicals for which there is some evidence for a SAR inducing effect include phosphates (K_2HPO_4, K_3PO_4, Na_3PO_4, Na_2HPO_4) and probenazole. In trials K_2HPO_4 reduced the incidence of potato late blight (*Phytophthora infestans*) by 87.1 % and increased the activities of chitinase and β-1,3-glucanase by 163 and 117 % respectively (Abd-El-Kareem *et al.* 2001). When sprayed onto cucumber leaves K_2HPO_4, K_3PO_4, Na_3PO_4, Na_2HPO_4 each induced systemic resistance to anthracnose caused by *Colletotrichum lagenarium* (Gottstein and Kuć 1989). Probenazole induces antifungal fatty acids such as α-linolenic acid and increases the activity of a range of enzymes involved in lignin biosynthesis including phenylalanine ammonia-lyase, catechol *o*-methyltransferase and peroxidase (Sekizawa and Mase 1981). Probenazole is available in Japan as Oryzemate (Meija Seika) where it is used for the control of rice blast (*Magnaporthe grisea*).

1.14.4 Induced systemic resistance

Plant pathogens and some strains of PGPR can induce resistance in plants. This type of induced resistance is known as 'induced systemic resistance'. PGPR mediated induced systemic resistance has been extensively reviewed by van Loon *et al.* (1998). These authors note that many of the PGPR that induce systemic resistance also have other modes of action against pathogens (eg. competition). Therefore, in order to establish ISR as the mechanism responsible for disease reduction the inducing

rhizobacteria and the pathogen must remain spatially separated for the duration of any experiment. According to this criterion some examples of ISR as the mode of disease suppression include: *P. fluorescens* WCS417 against Fusarium wilt of carnation (van Peer and Schippers 1992) and Fusarium wilt of tomato (Duijff *et al.* 1997), *Pseudomonas* spp. against *Pythium aphanidermatum* of cucumber (Zhou and Paulitz 1994), *Serratia marcescens* 90-166 against *F. oxysporum* f.sp. *cucumerinum* (Fusarium wilt of cucumber) (Liu *et al.* 1995), *Bacillus cereus* BS 03 and *Pseudomonas aeruginosa* RRLJ 04 against *Fusarium udum* (Fusarium wilt of pigeon pea) (Dutta *et al.* 2008).

A number of determinants of PGPR induced systemic resistance have been elucidated mainly using PGPR mutants, heat killed PGPR or purified components of PGPR. Using these mechanisms lipopolysaccharides (found in the outer membrane of the bacterium), siderophores or other iron regulated factors and salicylic acid (itself a bacterial siderophore) have demonstrated capacity to induce systemic resistance (van Loon *et al.* 1998).

PGPR trigger the production of jasmonic acid within the plant cell. Induction of the JA/ethylene signal transduction pathway leads to upregulation of plant defenses via ISR (Fig. 1.2). In addition, since many PGPR produce salicylic acid this bacterially produced SA can cause upregulation of plant defenses via systemic acquired resistance (SAR) and the salicylic acid pathway. This additional mechanism is active in plant species other than *Arabidopsis.*

ISR responses are also an important determinant of disease control mediated by some mycorrhizal fungi (Xavier and Boyetchko 2002) and endophytes (Kavroulakis *et al.* 2007).

1.15 Genetic engineering

There are numerous potential applications for genetic engineering in the management of soilborne plant disease. These include engineering the host to increase resistance to pathogen invasion or growth, engineering the pathogen to reduce virulence and engineering biological control agents to increase their efficacy or ability to compete with other soil microorganisms. In addition, genetic transformation techniques have been used by researchers to trace the movement of biocontrol strains and study population dynamics. Yeasts and *Trichoderma* sp. have been transformed with the green fluorescent protein (GFP) to track movement (Bae and Knudsen 2000; Nigro *et al.* 1999).

Genetic transformation has also been used to improve the potential of biological control agents. The ability of *T. harzianum* to colonise the root system of host plants has been improved by protoplast fusion. Harman (2000) fused a mutant strain capable of colonising plant roots with a strain that is able to compete with bacteria under iron limiting conditions. This new strain was better able to colonise the root system and provided better control of disease.

A novel approach used by Chen *et al.* (2000) involves engineering hypovirus strains responsible for hypovirulence of fungal pathogens. Using this approach these authors were able to uncouple small canker size and suppression of asexual sporulation in the chestnut blight fungus *Cryphonectria parasitica.* This work indicates the potential to enhance biological control by hypovirulent isolates of plant pathogenic fungi by balancing hypovirulence and ecological fitness.

1.16 Progress toward integrated control of soilborne plant pathogens

The impact of soilborne pathogens on vegetable production systems has increased over time due a range of factors including the overuse of crop monocultures and short rotations, declining soil organic matter and microbial diversity. Lovett (1982) points out that the short-term benefits of research into and use of agro-chemicals have been off-set by the development of longer-term problems, some of which may prove more intractable than those which they have superseded. Brader (1979) proposes that the realisation that single-method approaches would fail to solve insect pest problems took place in the early 1950s in North America, Europe and Australia. This was coupled with concern that sound ecological principles were not always practiced using agrochemicals and followed some years later with concern as to the impact of agrochemicals on non-target insect pests (Lovett 1982). These same concerns have been mirrored in the field of plant pathology, although some 5-10 years later, and the concept of integrated control has dominated the literature for at least 20 years since.

Problems associated with chemical cost, resistance development, lack of registrations, cost of registration, chemical withdrawal and deleterious effects on soil health have forced users to seek a broader perspective on the range of available control measures. Chemicals continue to form part of the solution; however increasingly specific fungicides (with a single mode of action) are replacing the

broad spectrum protectants. Spray numbers are being reduced through crop monitoring and 'decision support systems'; applications are becoming strategic rather than broadacre particularly targeting the root zones for soilborne diseases; chemical rotation is becoming more common in order to slow the development of resistance, and chemicals are being seen as one element of an overall management strategy.

This review has identified a range of potential, IPM compatible, non-chemical control options for the management of key soilborne pathogens affecting the Australian vegetable industry (Table 1.3). This information has been combined with the knowledge of local pathogen experts, compiled during a series of workshops conducted during late 2007 (Appendix 1.1), to produce proposed 'best practice' IPM strategies for *Sclerotinia, Pythium, Fusarium* and *Rhizoctonia* in Australia (Appendix 1.2). The effectiveness of many of the individual elements of each strategy are yet to be confirmed in Australia under Australian growing conditions and using Australian pathogen isolates and vegetable cultivars. They have been grouped together against the four modules of HAL pathology subprogram 2.2 (ie. eradicating pathogen survival structures, disrupting the infection process, strengthening the crop host against disease and exploiting host resistance) and served, in this format, as a plan for research conducted in the remaining years of the subprogram. Symbols denote work conducted in subprogram 2.2 and elsewhere in other subprograms or projects. In doing this the intent is not to replicate the good work already done or being done by others but to compliment it. The new structure within the HAL funded pathology program has promote collaboration and strengthened links and communication between these groups. If maintained this will lead more quickly towards the implementation of best-practice IPM strategies for the control of major soilborne diseases of vegetable crops.

Table 1.3 IPM compatible non-chemical control options for management of key soilborne pathogens of vegetable crops.

	Crop Rotation	Rouging	pH	Nutrition	Composts	Resistance and grafting	Surfactants and bio-surfactants	Plant volatiles	Biocontrol	Hypovirulence	SAR	ISR
Pythium			*P. sulcatum* (cavity spot) increase to 7.2	Increase Ca (cucumber and carrot) Increase Si (cucurbits, early application)			Particularly for zoosporic Pythium and hydroponic situations		Seed treatments of *P. oligandrum*	Non-transmissible hypovirulent strains eg. *P. oligandrum, P. nunn, P. mycoparasiticum, P. periplocum*	Chitin	*Pseudomonas* spp. eg. *P. aphanidermatum* of cucumber
Fusarium			*F. oxysporum* (cucurbits) increase to 6.5-7	N as NO_3^- rather than NH_4 Increase K (wilts) Increase B Increase Si (cucurbits)		Grafting - Cucurbits and snake bean (anything with low density plantings) where conidia of Fusarium not airborne Partially resistant basil available. Resistance in pea		Pepper/mustard, clove or cassia Crucifer derived ITCs		Non-transmissible hypovirulent strains of *F. oxysporum*	SA induced SAR Chitin BTH, BABA induced SAR	*P. fluorescence* against Fusarium wilt *Serratia marcescens* against Fusarium wilt
Sclerotinia	Broccoli as preceeding crop Biofumigant break crops	Of lettuce crops to prevent inoculum build up				Selection of bean varieties for low canopy density, upright stature		Oregano Fennel Crucifer derived ITCs	*Sporidesmium sclerotivorum,* low dose after harvest before discing. *C. minitans* immediately after harvest	Transmissible hypovirulent strains of *S. minor* and *S. homeocarpa,* NOT *S. sclerotiorum* as it has many vegetative compatibility groups	SA induced SAR INA, BTH induced SAR	
Rhizoctonia				N as NO_3^- rather than NH_4 Increase P Increase S Increase B						Non transmissible hypovirulent strains of *R. solani* and *P. oligandrum*	SA induced SAR Chitin	
General	Rotation/integrated cropping systems/reduced tillage for soil health, biological activity				Addition to nursery mixes to suppress damping off diseases				At harvest applications of some BCAs to enable pathogen to increase on organic matter and parasitise propagules on crop (eg. *Trichoderma* and P. nunn)		SAR	

1.17 Acknowledgements

The authors gratefully acknowledge the assistance of Jacqueline Edwards (DPI VIC), Len Tesoriero (NSW DPI) and Barbara Hall (SARDI) for reviewing the manuscript.

In addition the assistance of 'pathogen experts' who participated in the various pathogen specific workshops during late 2007 is gratefully acknowledged. Their knowledge of the individual pathogens and control measures currently being implemented is invaluable and their practical contributions to this review cannot be underestimated. We are indebted to them all for their participation and willingness to openly contribute their research findings.

Sclerotinia workshop
Oscar Villalta (DPI Vic), Denise Wite (DPI Vic), Calum Wilson (UTAS), John Duff (QDPI & F), Garry McNab (Simplot), Sue Cross (Bayer), Doug Wilson (NuFarm), Rob Velthius (Xeron), Ian Porter (DPI Vic) , Caroline Donald (DPI Vic), Barry Kerr (Sumitomo), Alicia Greenhill (La Trobe Uni), Rebecca Barnett (La Trobe Uni), Kim Plummer (La Trobe Uni), Hoong Pung (Peracto), Suzie Jones (UTAS), Frank Hay(TIAR/UTAS), Sarah Pethybridge (TIAR/UTAS), David Gent(USA) , Philip Frost (Peracto)

Pythium & Fusarium workshop
Graham Stirling (Biological Crop Protection), David Nehl (NSW DPI), Christine Horlock (QLD DPI & F), Edward Leiw (Botanic Gardens) , Leanne Forsyth (NSW DPI), Liz Minchinton (VIC DPI), Andrew Watson (NSW DPI), Ameera Youseph (Syd Uni), Des Auer (VIC DPI), Luc Streit (Syngenta), Rob Velthius (Xeron), Len Tesoriero (NSW DPI), Ian Porter (VIC DPI), Caroline Donald (VIC DPI), Barry Conde (NT DPIFM), Barbara Hall (SARDI), Trevor Klein(Syngenta), Peter Dal Santo (Agaware)

Rhizoctonia workshop
Dolf deBoer (DPI VIC), Jon McCarthy (BarMac), Susan Cross (Bayer), Alistar Beyer (Bayer), Peter dal Santo (Agaware), Rob Velthuis (Xeron), Scott Mattner (DPI), Ken Labbett (PPIAC), Iain Kirkwood (PPRD), Chris Russell (Simplot), James Hills (Agronico), Mark Atkinson (Syngenta), Trevor Stebbings (Syngenta), Hoong Pung (Peracto), Chris Van der Hoven (Sumitomo), Barry Ker (Sumitomo), Andrew Watson (Agnova Technologies), Ian Fraser, Michael Tool (Yield Enhancement Services), Kathy Ophel-Keller (SARDI), Neville Fernando (DPI), Barbara Hall (SARDI), Cathy Todd (Uni Adelaide), Ian Porter (DPI), Cassie Scoble (DPI), Andrew Pittman (Crop and Food NZ), Peter Lane (Lane Bros Warnambool VIC), Caroline Donald (DPI), Luc Streit (Syngenta), Phil Jobling.

1.18 References

Abd-El-Kareem F, Abd-Alla MA, El-Mohamedy RSR (2001) Induced resistance in potato plants for controlling late blight disease under field conditions. *Egyptian Journal of Phytopathology* **29**, 29-41.

Abd-El-Kareem F, El-Mougy NS, El-Gamal NG, Fotouh YO (2006) Use of chitin and chitosan against tomato root rot disease under greenhouse conditions. *Research Journal of Agriculture and Biological Sciences* **2**, 147-152.

Adams PB (1989) Comparison of antagonists of *Sclerotinia* species. *Phytopathology* **79**, 1345-1347.

Adams PB (1990) The potential of mycoparasites for biological control of plant diseases. *Annual Review of Phytopathology* **28**, 59-72.

Adams PB, Ayers WA (1979) Ecology of *Sclerotinia* species. *Phytopathology* **69**, 896-899.

Adams PB, Fravel DR (1990) Economical biological control of Sclerotinia lettuce drop by *Sporidesmium sclerotivorum*. *Phytopathology* **80**, 1120-1124.

Addepalli MK, Fujita Y (2002) Regulatory role of external calcium on *Pythium porphyrae* (Oomycota) zoospore release, development and infection in causing red rot disease of *Porphyra yezoensis* (Rhodophyta). *FEMS Microbiology Letters* **211**, 253-257.

Al-Sa'di AM, Drenth A, Deadman ML, Al-Said FA, Khan I, Aitken EAB (2008) Association of a second phase of mortality in cucumber seedlings with a rapid rate of metalaxyl biodegradation in greenhouse soils. *Crop Protection* **27**, 1110-1117.

Alabouvette C, Couteaudier Y, Louvet J (1984) Recherches sur la résistance des sols aux maladies. X. Comparaison de la mycoflore colonisant les raciness de melons cultivés dans un sol resistant ou dans un sol sensible aux fusarioses vasculaires. *Agronomie* **4**, 735-740.

Andresen L, Becker W, Schlüter K, Burges J, Parthier B, Apel K (1992) The identification of leaf thionin as one of the main jasmonate-induced proteins of barley (*Hordeum vulgare*). *Plant Molecular Biology* **19**, 193-204.

Anilkumar TB, Pandourange Gowda KT (1981) Growth and stability of penta chloronitro benzene resistant isolate of *Rhizoctonia solani*. *Zentralblatt fuer Bakteriologie Parasitenkunde Infektionskrankheiten und Hygiene Zweite Naturwissenschaftliche Abteilung Mikrobiologie der Landwirtschaft der Technologie und des Umweltschutzes* **136**, 502-504.

Anon Fusarium Diseases. Available at http://www.rbgsyd.nsw.gov.au/science/hot_science_topics/Soilborne_plant_diseases/Vietnam_template3/Fusarium [Verified 14 May, 2008]

APVMA Australian Pesticides and Veterinary Medicines Authority. Available at http://www.apvma.gov.au/index.asp [Verified 10th May, 2008]

Arora NK, Kim MJ, Kang SC, Maheshwari DK (2007) Role of chitinase and β-1,3-glucanase activities produced by a fluorescent pseudomonad and in vitro inhibition of *Phytophthora capsici* and *Rhizoctonia solani*. *Canadian Journal of Microbiology* **53**, 207-212.

Asirifi K-N, Morgan WC, Parbery DG (1994) Suppression of sclerotinia soft rot of lettuce with organic soil amendments. *Australian Journal of Experimental Agriculture* **34**, 131-136.

Avenot HF, Michailides TJ (2007) Resistance to boscalid fungicide in *Alternaria alternata* isolates from pistachio in California. *Plant Disease* **91**, 1345-1350.

Bae YS, Knudsen GR (2000) Co-transformation of *Trichoderma harzianum* with β-glucuronidase and green fluorescent protein genes provides a useful tool for monitoring fungal growth and activity in natural soils. *Applied and Environmental Microbiology* **66**, 810-815.

Bailey AM, Coffey MD (1985) Biodegredation of metalaxyl in avocado soils. *Phytopathology* **75**, 135-137.

Bailey KL, Duczek LJ (1996) Managing cereal diseases under reduced tillage. *Canadian Journal of Plant Pathology* **18**, 159-167.

Bailey KL, Lazarovits G (2003) Suppressing soil-borne diseases with residue management and organic amendments. *Soil and Tillage Research* **72**, 169-180.

Bardin SD, Huang HC (2001) Research on biology and control of *Sclerotinia* diseases in Canada. *Canadian Journal of Plant Pathology* **23**, 88-89.

Bastels-Schooley J, MacNeil BH (1971) A comparison of the mode of action of three benzimidazoles. *Phytopathology* **61**, 816-819.

Bélanger RR, Bowen PA, Ehret DL, Menzies JG (1995) Soluble silicon: its role in crop and disease managment of greenhouse crops. *Plant Disease* **79**, 329-336.

Bell AA, Hubbard JC, Lui L, Davis RM, Subbaroa KV (1998) Effects of chitin and chitosan on the incidence and severity of Fusarium yellows of celery. *Plant Disease* **82**, 322-328.

Benhamou N, Bélanger R (1998) Benzothiadiazole-mediated induced resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato. *Plant Physiology* **118**, 1203-1212.

Benhamou N, Kloepper JW, Tuzun S (1998) Induction of resistance against Fusarium wilt of tomato by combination of chitosan with and endophytic bacterial strain: ultrastructure and cytochemistry of the host response. *Planta* **204**, 153-168.

Bianchi A, Zambonelli A, Zechini D'Aulerio A, Bellesia F (1997) Ultrastructural studies of the effects of *Allium sativum* on phytopathogenic fungi in vitro. *Plant Disease* **81**, 1241-1246.

Bletsos FA (2006) Grafting and calcium cyanamide as alternatives to methyl bromide for greenhouse eggplant production. *Scientia Horticulturae* **107**, 325-331.

Bletsos FA, Thanassoulopoulos CC, Roupakias DG (2003) Effect of grafting on growth, yield, and verticilium wilt of eggplant. *HortScience* **38**, 183-186.

Bockus WW, Shroyer JP (1998) The impact of reduced tillage on soilborne plant pathogens. *Annual Review of Phytopathology* **36**, 485-400.

Boland GJ (1992) Hypovirulence and double-stranded RNA in *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology* **14**, 10-17.

Boland GJ (2004) Fungal viruses, hypovirulence, and biological control of *Sclerotinia* species. *Canadian Journal of Plant Pathology* **26**, 6-18.

Boland GJ, Leggett F, Kokko E (2000) Ultrastructural comparisons of hypovirulent and virulent isolates of *Sclerotinia homoeocarpa*. *Canadian Journal of Plant Pathology* **22**, 181 (abstract).

Bowers JH, Locke JC (2000) Effect of botanical extracts on the population density of *Fusarium oxysporum* in soil and control of fusarium wilt in the greenhouse. *Plant Disease* **84**, 300-305.

Brader L (1979) Integrated pest control in the developing world. *Annual Review of Entomology* **24**, 225-254.

Brenneman TB, Phipps PM, Stipes RJ (1987) Sclerotinia blight of peanut relationship between *in vitro* resistance and field efficacy of dicarboximide fungicides. *Phytopathology* **77**, 1028-1032.

Broadbent P, Baker KF, Waterworth Y (1971) Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils. *Australian Journal of Biological Science* **24**, 925-944.

Brooker NL, Long JH, Collins J (2004) Plant lipid derivative seed treatments for managing fungal soybean diseases. In '16th International Plant Lipid Symposium'. Budapest, Hungary pp. 149-151. (Hungarian Scientific Society for Food Industry (MÉTE) and the Budapest University of Economic Sciences and Public Administration Faculty of Food Science)

Brožová J (2002) Exploitation of the mycoparasitic fungus *Pythium oligandrum* in plant protection. *Plant Protection Science* **38**, 29-35.

Butt TM, Copping LG (2000) Fungal biological control agents. *Pesticide Outlook* **October 2000**, 186-191.

Campbell RN, Greathead AS, Myers DF, de Boer GJ (1985) Factors related to control of clubroot of crucifers in the salinas valley of California. *Phytopathology* **75**, 665-670.

Cardoso JE, Echandi E (1987) Nature of protection of bean seedlings from Rhizoctonia root rot by a binucleate *Rhizoctonia*-like fungus. *Phytopathology* **77**, 1548-1511.

Caron M, Fortin JA, Richard C (1986) Effect of *Glomus intraradices* on infection by *Fusarium oxysporum* f.sp. *radicis-lycopersici* in tomatoes over a 12-week period. *Canadian Journal of Botany* **64**, 552-556.

Cavallito CJ, Bailey HJ (1944) Allicin, the antibacterial principle of *Allium sativum* I. Isolation, physical properties and antibacterial action. *Journal of the American Chemistry Society* **66**, 1950-1951.

Chang C, Meyerowitz EM (1995) The ethylene hormone response in *Arabidopsis:* A eukaryotic two-component signalling system. *Proceedings of the National Academy of Science USA* **92**, 4129-4133.

Chang W-T, Chen Y-C, Jao C-L (2007) Antifungal activity and enhancement of plant growth by *Bacillus cereus* grown on shellfish chitin wastes. *Bioresource Technology* **98**, 1224-1230.

Charles P (2006) DMDS: a new alternative for soil disinfestation. In '2006 Annual International Research Conference on Methyl Bromide'. (San Diego, CA)

Chen B, Geletka LM, Nuss DL (2000) Using chimeric hypoviruses to fine-tune the interaction between a pathogenic fungus and its plant host. *Journal of Virology* **74**, 7562-7567.

Chen C, Bélanger RR, Benhamou N, Paulitz TC (1999) Role of salicylic acid in systemic resistance induced by *Pseudomonas* spp. against *Pythium aphanidermatum* in cucumber roots. *European Journal of Plant Pathology* **105**, 477-486.

Cheng Y, McNally DJ, Labbé C, Voyer N, Belzile F, Bélanger RR (2003) Insertional mutagenesis of a fungal biocontrol agent led to discovery of a rare cellobiose lipid with antifungal activity. *Applied and Environmental Microbiology* **69**, 2595-2602.

Cherif M, Asselin A, Belanger RR (1994) Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. *Phytopathology* **84**, 236-242.

Cherif M, Bélanger RR (1992) Use of potassium silicate amendments in recirculating nutrient solutions to suppress *Pythium ultimum* on long English cucumber. *Plant Disease* **76**, 1008-1011.

Chester KS (1933) The problem of acquired physiological immunity in plants. *The Quarterly Review of Biology* **8**, 275-324.

Chitarra GS, Breeuwer P, Nout MJR, van Aelst AC, Rombouts FM, Abee R (2003) An antifungal compound produced by *Bacillus subtillis* YM 10-20 inhibits germination of *Penicillium roqueforti* conidiospores. *Journal of Applied Microbiology* **94**, 159-166.

Cho CW, Fuller MS (1989) Observations of the water expulsion vacoule of Phytophthora palmivora. *Protoplasma* **149**, 47-55.

Choi D, Bostock RM, Avdiushko S, Hildebrand DF (1994) Lipid-derived signals that discriminate wound- and pathogen- responsive isoprenoid pathways in plants: Methyl jasmonate and the fungal elicitor arachidonic acid induce different 3-hydroxy-3-methylglutaryl-coenzyme A reductase genes and antimicrobial isoprenoids in *Solanum tuberosum* L. *Proceedings of the National Academy of Science USA* **91**, 2329-2333.

Cichy KA, Snapp SS, Kirk WW (2007) Fusarium root rot incidence and root system architecture in grafted common bean lines. *Plant Soil* **300**, 233-244.

Cohen R, Horev C, Burger Y, Shriber S, Hershenhorn J, Katan J, Edelstein M (2002) Horticultural and pathological aspects of fusarium wilt management using grafted melons. *HortScience* **37**, 1069-1073.

Cohen Y, Gisi U, Niderman T (1993) Local and systemic protection against Phytophthora infestans induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. *Phytopathology* **83**, 1054-1062.

Colbert SF, Hendson M, Ferri M, Schroth MN (1993a) Enhanced growth and activity of a biocontrol bacterium genetically engineered to utilize salicylate. *Applied and Environmental Microbiology* **59**, 2017-2076.

Colbert SF, Schroth MN, Weinhold AR, Hendson M (1993b) Enhancement of population densities of *Pseudomonas putida* PpG7 in agricultural ecosystems by selective feeding with the carbon source salicylate. *Applied and Environmental Microbiology* **59**, 2064-2070.

Cole H, Warren CG, Sanders PL (1974) Fungicide tolerance - a rapidly emerging problem in turfgrass disease control. In 'Second International Turfgrass Research Conference'. Madison, Wisconsin. (Ed. EC Roberts) pp. 344-349. (American Society of Agronomy)

Colhoun J (1958) Club root disease of brassicas caused by Plasmodiophora brassicae Woron. Phytopathological Paper. Commonwealth Mycological Institute, Kew.

Compant S, Duffy B, Nowak J, Clément C, Ait Barka E (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology* **71**, 4951-4959.

Condé B, Aeo-Aro I, Pitkethley R (2002) Grafting snake beans to control fusarium wilt. Agnote No. 807. (Ed. Agdex No. 258/622). Northern Territory Department of Primary Industries.

Condé B, Arao-Arao I, R. Pitkethley R (2005) How to Control Fusarium Wilt and Base Rot in Sweet Basil. Information Sheet No. 14. Northern Territory Government, Department of Primary Industry, Fisheries and Mines.

Conn KL, Lazarovits G (1999) Impact of aminal manures on verticillium wilt, potato scab, and soil microbial populations. *Canadian Journal of Plant Pathology* **21**, 81-92.

Conn KL, Lazarovits G (2000) Soil factors influencing the efficacy of liquid swine manure added to soil to kill *Verticillium dahliae*. *Canadian Journal of Plant Pathology* **22**, 400-406.

Copping LG (2004) 'The manual of biocontrol agents.' (British Crop Protection Council: Alton, Hampshire)

Corden ME (1965) Influence of calcium nutrition on *Fusarium* wilt of tomato and polygalacturonase activity. *Phytopathology* **55**, 222-224.

Cowan M (1999) Plant products as anti-microbial agents. *Clinical Microbiology Reviews* **10**, 564-582.

Craft CM, Nelson EB (1996) Microbial properties of composts that suppress damping-off and root rot of creeping bentgrass by *Pythium graminicola*. *Applied and Environmental Microbiology* **62**, 1550-1557.

Creelman RA, Mullet JE (1995) Jasmonic acid distribution and action in plants: Regulation during development and response to biotic and abiotic stress. *Proceedings of the National Academy of Science USA* **92**, 4114-4119.

Creelman RA, Tierney ML, Mullet JE (1992) Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene expression. *Proceedings of the National Academy of Science USA* **89**, 4938-4941.

Crump NS, Donald EC, Wiechel TJ, Scoble C, O'Toole AK (2007) The use of organic amendments for the control of powdery scab (*Spongospora subterranea*) of potato. In 'Proccedings of the 16th Biennial Australasian Plant Pathology Society Conference Back to Basics: Managing Plant Disease'. Adelaide p. 125.

Curtis H, Noll U, Störmann J, Slusarenko AJ (2004) Broad-specturm activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and oomycetes. *Physiological and Molecular Plant Pathology* **65**, 79-89.

Dann E, Diers B, Byrum J, Hammerschmidt R (1998) Effect of treating soybean with 2,6-dichloro-isonicotinic acid (INA) and benzothiadizole (BTH) on seed yields and the level of disease caused by *Sclerotinia sclerotiorum* in field and greenhouse studies. *European Journal of Plant Pathology* **104**, 271-278.

Darby HM, Stone AG, Dick RP (2006) Compost and manure mediated impacts on soilborne pathogens and soil quality. *Soil Biology and Biochemistry* **70**, 347-358.

Datnoff LE, Elmer WH, Huber DM (2007) 'Mineral nutrition and plant disease.' (The American Phytopathological Society: St. Paul, Minnesota)

Davanlou M, Madsen AM, Masden CH, Hockenhull J (1999) Parasitism of macroconidia, chlamydospores and hyphae of *Fusarium culmorum* by mycoparasitic *Pythium* species. *Plant Pathology* **48**, 352-359.

Davison E, McKay A (1999) Cavity spot disease of carrots. Farmnote. Agriculture Western Australia.

Davison EM, McKay AG (2003) Host range of Pythium sulcatum and the effects of rotation on Pythium diseases of carrots. *Australasian Plant Pathology* **32**, 339-346.

De Jonghe K, De Dobbelaere I, Sarrazyn R, Höfte M (2005) Control of brown root rot caused by *Phytophthora cryptogea* in the hydroponic forcing of witlof chicory (*Chichorium intybus* var. *foliosum*) by means of a nonionic surfactant. *Crop Protection* **24**, 771-778.

De Jonghe K, Hermans D, Höfte M (2007) Efficacy of alcohol alkoxylate surfactants differing in the molecular structure of the hydrophilic portion to control *Phytophthora nicotianae* in tomato substrate culture. *Crop Protection* **26**, 1524-1531.

Dehne HW, Schönbeck F (1979) Investigations on the influence of endotrophic mycorrhiza on plant diseases. II. Phenol metabolism and lignification. *Phytopathologische Zeitschrift* **95**, 210-216.

del Rio LE, Gulya T, Lamppa RS, Balbysheb N (2004) Incidence of Sclerotinia mycoparasites across the midwest and implications for the use of Intercept. In '26th Sunflower Research Workshop'. Fargo, ND

Deng F, Xu R, Boland GJ (2003) Hypovirulence associated double-stranded RNA from *Sclerotinia homoeocarpa* is conspecific with *Ophiostoma novu-ulmi* mitovirus 3a-Ld. *Phytopathology* **93**, 1407-1414.

Detweiler AR, Yargas JMJ, Danneberger TK (1983) Resistance of *Sclerotinia homoeocarpa* to iprodione and benomyl. *Plant Disease* **67**, 627-630.

DeVleeschauwer D, Verdonck O, Van Assche P (1981) Phytotoxicity of refuse compost. *BioCycle* **2**, 44-46.

Diánez F, Santos M, Tello JC (2005) Suppression of soilborne pathogens by compost: suppressive effects of grape marc compost on phytopathogenic oomycetes. *Acta Horticulturae* **697**, 441-460.

Dixon GR, Webster MA (1988) Antagonistic effects of boron, calcium and pH on pathogenesis caused by *Plasmodiophora brassicae* Woronin (clubroot) - a review of recent work. *Crop Research (Horticultural Research)* **28**, 83-95.

Doares SH, Syrovets T, Weiler EW, Ryan CA (1995) Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway. *Proceedings of the National Academy of Science USA* **92**, 4095-4098.

Donald EC, Lawrence JM, Porter IJ (2004) Influence of particle size and application method on the efficacy of calcium cyanamide for control of clubroot of vegetable brassicas. *Crop Protection* **23**, 297-303.

dos Santos I, Bettiol W (2003) Effect of sewage sludge on the rot and seedling damping-off of bean plants caused by *Sclerotium rolfsii*. *Crop Protection* **22**, 1093-1097.

Duff J, Pitkethley R (2003) Soil solarisation. Agnote No. 537. Northern Territory Department of Primary Industries.

Duff JD, Barnaart A (1992) Solarisation controls soilborne fungal pathogens in nursery potting mixes. *Australasian Plant Pathology* **21**, 20-23.

Duffy BK, Défago G (1999) Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. *Applied and Environmental Microbiology* **65**, 2429-2438.

Duijff BJ, Gianinazzi-Pearson V, Lemanceau P (1997) Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol *Pseudomonas fluorescens* strain WCS417r. *New Phytologist* **135**, 325-334.

Duijff BJ, Meijer JW, Bakker PAHM, Schippers B (1993) Siderophore-mediated competicion for iorn and induced resistance in the suppression of fusarium wilt of carnation by fluorescent *Pseudomonas* spp. *Netherlands Journal of Plant Pathology* **99**, 277-289.

Durrant WE, Dong X (2004) Systemic acquired resistance. *Annual review of Phytopathology* **42**, 185-209.

Dutkey EM Fungicides and other plant disease management approaches. Available at http://www.colonialdistrictroses.org/sitebuildercontent/sitebuilderfiles/fungicidesand otherdisease.pdf [Verified 22 Feb 2008]

Dutta S, Mishra AK, Dileep Kumar BS (2008) Induction of systemic resistance against fusarial wilt in pigeon pea through interaction of plant growth promoting rhizobacteria and rhizobia. *Soil Biology and Biochemistry* **40**, 452-461.

Edelstein M (2004) Grafting vegetable-crop plants: pros and cons. *Acta Horticulturae* **659**, 235-238.

El-Kazzaz AA, El-Mougy NS (2001) Inheritance of disease resistance in cucumber plants to root rot caused by *Fusarium solani* using tissue culture techniques. *Egyptian Journal of Phytopathology* **29**, 57-68.

El-Mougy NS (2002) *In vitro* studies on antimicrobial activity of salicylic acid and acetylsalicylic acid as pesticidal alternatives against some soilborne pathogens. . *Egyptian Journal of Phytopathology* **30**, 41-55.

El-Mougy NS (2004) Preliminary evaluation of salicylic acid and acetylsalicylic acid efficacy for controlling root rot diseases of lupin under greenhouse conditions. *Egyptian Journal of Phytopathology* **32**, 11-21.

El Ghaouth A, Arul J, Benhamou N, Asselin N, Belanger NN (1994) Effect of chitosan on cucumber plants: Suppression of *Pythium aphanidermatum* and induction of defense reactions. *Phytopathology* **84**, 313-320.

El Hassni M, El Hadrami A, Daayf F, Ait Barka E, El Hadrami I (2004) Chitosan, antifungal product against *Fusarium oxysporum* f. sp. *albedinis* and elicitor of defence reactions in date palm roots. *Phytopathologia Mediterranea* **43**, 195-204.

Elad Y, Chet I, Henis Y (1982) Degradation of plant pathogenic fungi by *Trichoderma harzianum*. *Canadian Journal of microbiology* **28**, 719-725.

Elliston JE (1982) Hypovirulence. *Advances in Plant Pathology* **1**, 1-33.

Epstein E (1972) 'Mineral nutrition of plants: principles and perspectives.' (John Wiley and sons: New York.)

Farmer EE, Ryan CA (1990) Interplant communication: Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings of the National Academy of Science USA* **87**, 7713-7716.

Fauteux F, Chain F, Belzile F, Menzies JG, Bélanger RR (2006) The protective role of silicon in the *Arabidopsis*-powdery mildew pathosystem. *Proceedings of the National Academy of Science USA* **103**, 17554-17559.

Fauteux F, Rémus-Borel W, Menzies JG, Bélanger RR (2005) Silicon and plant disease resistance against pathogenic fungi. *FEMS Microbiology Letters* **249**, 1-6.

Fobert PR, Després C (2005) Redox control of systemic acquired resistance. *Current Opinion in Plant Biology* **8**, 378-382.

Forsyth LM, Carrus R, Bertus F, Tesoriero LA (2007a) New control methods for fusarium wilt of cucumber. In '16th Biennial Australasian Plant Pathology Conference'. Adelaide, South Australia p. 115

Forsyth LM, Hamill SD, Aitken EAB, Smith LJ (2007b) A role for silicon in resistance to fusarium wilt. In '16th Biennial Australasian Plant Pathology Conference'. Adelaide, South Australia p. 146

FRAC (2006) FRAC list of plant pathogenic organisms resistant to disease control agents. Available at http://frac.info.org [Verified 18th Feb, 2008]

FRAC (2007) FRAC Code List: Fungicides sorted by mode of action. Available at http://frac.info.org [Verified 22nd Feb, 2008]

Fravel DR (2007) Lessons learned from *Sporidesmium,* a fungal agent for control of sclerotia-forming fungal pathogens. In 'Biological control a global perspective'. (Eds C Vincent, MS Goettel and G Lazarovits) pp. 218-223. (CAB International/ AAFC: Oxfordshire, UK)

Fridlender M, Inbar J, Chet I (1993) Biological control of soilborne plant pathogens by a β-1,3-glucanase-producing *Pseudomonas cepacia*. *Soil Biology and Biochemistry* **25**.

Gamliel A, Katan T, Yunis H, Katan J (1996) Fusarium wilt and crown rot of sweet basil: involvement of soilborne and airborne inoculum. *Phytopathology* **86**, 56-62.

Gil SV, Haro R, Oddino C, Kearney M, Zuza M, Marinelli A, March GJ (2008) Crop management practices in the control of peanut diseases caused by soilborne fungi. *Crop Protection* **27**, 1-9.

Gisi U, Sierotzki H, Cook A, McCaffery A (2002) Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. *Pest Management Science* **58**, 859-867.

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual review of Phytopathology* **43**, 205-277.

Godoy G, Rodriguez KR, Morgan JG (1983) Chitin amendment for control of *Meloidogyne arenaria* in infested soil. 2: Effects of microbial population. *Nematropica* **12**, 71-84.

Gottstein HD, Kuć JA (1989) Induction of systemic resistance to anthracnose in cucumber by phosphates. *Phytopathology* **79**, 176-179.

Grant M, Lamb C (2006) Systemic immunity. *Current Opinion in Plant Biology* **9**, 414-420.

Grinstein A, Katan J, Abdul-Razik A, Zeydan O, Elad Y (1979) Control of Sclerotium rolfsii and weeds in peanuts by solar heating of soil. *Plant Disease Reporter* **63**, 1056-1059.

Gubler F, Jablonsky PP, Duniec J, Hardham AR (1990) Localization of calmodulin in flagella of zoospores of Phytophthora cinnamomi. *Protoplasma* **155**, 233-238.

Gundlach H, Müller MJ, Kutchan TM, Zenk MH (1992) Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. *Proceedings of the National Academy of Science USA* **89**, 2389-2393.

Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biocontrol of plant disease. *Annual Review of Phytopathology* **41**, 117-153.

Hammond-Kosack KE, Jones JDG (1997) Plant resistance genes. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 575-607.

Haneklaus S, Bloem E, Schnug E (2007) Sulfur and Plant Disease. In 'Mineral Nutrition and Plant Disease'. (Eds LE Datnoff, WH Elmer and DM Huber) pp. 101-118. (The American phytopathological Society: St. Paul, Minnesota)

Hanson LE, Schwager SJ, Loria R (1996) Sensitivity to thiabendazole in Fusarium species associated with dry rot in potatoes. *Phytopathology* **86**, 378-384.

Hardham AR (1992) Cell biology of pathogenesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**, 491-526.

Harman GE (2000) Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzinum* T-22. *Plant Disease* **84**, 377-393.

Harrison DJ, Burgess PG (1962) Use of rootstock resistance for controlling fusarium wilt of tomatoes. *Plant Pathology* **11**, 23-25.

He SS, Zhang BX, Ge QX (1992) On the antagonism by hyperparasite *Pythium oligandrum*. *Acta Phytopathologica Sinica* **22**, 77-82.

Heine G, Tikum G, Horst WJ (2006) The effect of silicon on the infection by and spread of *Pythium aphanidermatum* in single roots of tomato and bitter gourd. *Journal of experimental Botany* **58**, 569-577.

Hering TF, Cook RJ, Tang WH (1987) Infection of wheat embryos by Pythium species during seed germination and the influence of seed age and soil matric potential. *Phytopathology* **77**, 1104-1108.

Hjort K, Lembke A, Speksnijder A, Smalla K, Jansson JK (2007) Community structure of actively growing bacterial populations in plant pathogen suppressive soil. *Microbial Ecology* **53**, 399-413.

Hoes JA, Huang HC (1985) Effect of between-row and within-row spacings on development of sclerotinia wilt and yield of sunflower. *Canadian Journal of Plant Pathology* **7**, 98-102.

Hointink HAJ, Inbar Y, Boehm MJ (1991) Status of compost-amended potting mixes naturally suppressive to soilborne diseases of floricultural crops. *Plant Disease* **75**, 869-873.

Hoitink HAJ, Fahy PC (1986) Basis for the control of soilborne plant pathogens with composts. *Annual Review of Phytopathology* **24**, 93-114.

Hoitink HAJ, Stone AG, Han DY (1997) Suppression of plant diseases by composts. *HortScience* **32**, 184-187.

Horiuchi S, Hori M, Takashi S, Shimizu K (1982) Factors responsible for the development of clubroot supressing effect in soil solarization. *Bulletin of the Chugoku National Agricultural Experimental Station* **E 20**, 25-48.

Howell CR (2003) Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Disease* **87**, 4-10.

Huang HC (1977) Importance of *Coniothyrium minitans* in survival of sclerotia of *Sclerotinia sclerotiorum* in wilted sunflower. *Canadian Journal of Botany* **55**, 289-295.

Huang HC (1980) Control of Sclerotinia wilt of sunflower by hyperparasites. *Canadian Journal of Plant Pathology* **2**, 26-32.

Huang HC, Kemp GA (1989) Growth habit of dry beans (*Phaseolus vulgaris*) and incidence of white mould *(Sclerotinia sclerotiorum*). *Plant Protection Bulletin* **31**, 304-309.

Huang HC, Kokko EG (1988) Penetration of hyphae of *Sclerotinia sclerotiorum* by *Coniothyrium minitans* without the formation of appressoria. *Journal of Phytopathology* **123**, 133-139.

Huang JW, Kuhlman EG (1991) Mechanisms inhibiting damping-off pathogens of slash pine seedlings with a formulated soil amendment. *Phytopathology* **81**, 171-177.

Hubbard JC, Subbarao KV, Koike ST (1997) Development and significance of dicarboximide resistance in *Sclerotinia minor* isolates from commercial lettuce fields in California. *Plant Disease* **81**, 148-153.

Huber DM (1991) The use of fertilizers and organic amendments in the control of plant disease. In 'CRC Handbook of pest managment in agriculture'. (Ed. D Pimentel) pp. 169-204. (CRC Press: Boca Raton, Fla.)

Huber DM, Thompson IA (2007) Nitrogen and plant disease. In 'Mineral nutrition and plant disease'. (Eds LE Datnoff, WH Elmer and DM Huber) pp. 31-44. (The Americal Phytopathological Society: St Paul, Minnesota)

Huber DM, Watson RD (1974) Nitrogen form and plant disease. *Annual Review of Phytopathology* **12**, 139-165.

Isnaini M, Keane PJ (2007) Biocontrol and epidemiology of lettuce drop caused by *Sclerotinia minor* at Bacchus Marsh, Victoria. *Australasian Plant Pathology* **36**, 295-304.

Jeffery S, Williams E, Whicker C, Khoo C, Summerell B, Offord C, Fahy P, Noble D (1997) Some effects of silicate on *Pythium* damping-off of cucumbers in potting mixes. *Acta Horticulturae* **450**, 467-474.

Jocobsohn R, Greenberger A, Katan J, Levi M, Alon H (1980) Control of Egyptian broomrape (*Orobanche aegyptiaca*) and other weeds by means of solar heating of the soil by polyethylene mulching. *Weed Science* **28**, 312-316.

Jones EE, Deacon JW (1995) Comparative physiology and behaviour of the mycoparasites *Pythium acanthophoron, P. oligandrum* and *P. mycoparasiticum*. *Biocontrol Science and Technology* **5**, 27-39.

Jones EE, Stewart A (2000) Selection of mycoparasites of sclerotia of *Sclerotinia sclerotiorum* isolated from New Zealand soils. *New Zealand Journal of Crop and Horticultural Science* **28**, 105-114.

Kamensky M, Ovadis M, Chet I, Chernin L (2003) Soil-borne strain of IC14 of *Serratia plymuthica* with multiple mechanisms of antifungal activity provides biocontrol of *Botrytis cinerea* and *Sclerotinia sclerotiorum* diseases. *Soil Biology and Biochemistry* **35**, 323-331.

Karim R, Rajotte E, Heinrichs EA (2003) Eggplant grafting: a boon to Bangladeshi farmers. IPM CRS Progress Report No. 1. IPM CRSP Office of International Research, Education and Development, Virginia.

Karling JS (1968) 'The Plasmodiophorales.' (Hafner Publishing Company: New York)

Katagiri F (2004) A global view of defense gene expression regulation - a highly interconnected signalling network. *Current Opinion in Plant Biology* **7**, 506-511.

Katan J (1980) Solar Pasteurization of soils for disease control: Status and Prospects. *Plant Disease* **64**, 450-454.

Katan J (1981) Solar heating (solarization) of soil for control of soilborne pests. *Annual Review of Phytopathology* **19**, 211-236.

Katan J, Greenberger A, Alon H, Grinstein A (1976) Solar heating by polyethylene mulching for the control of diseases caused by soil-borne pathogens. *Phytopathology* **66**, 683-688.

Katan T, Shlevin E, Katan J (1997) Sporulation of *Fusarium oxysporum* f. sp. *lycopersici* on stem surfaces of tomato plants and aerial dissemination of inoculum. . *Phytopathology* **87**, 712-719.

Kavroulakis N, Spyridon N, Zervakis GI, Ehaliotis C, Haralampidis K, Papadopoulou KK (2007) Role of ethylene in the protection of tomato plants against soil-borne fungal pathogens conferred by an endophytic *Fusarium solani* strain. *Journal of Experimental Botany* **58**, 3853-3864.

Kilic O, Griffin GJ (1998) Effect of dsRNA-containing and dsRNA-free hypovirulent isolates of *Fusarium oxysporum* on severity of Fusarium seedling disease of soybean in naturally infested soil. *Plant and Soil* **201**, 125-135.

Kilikocka H, Haneklaus S, Bloem E, Schnug E (2005) Influence of sulfur fertilization on infection of potato tubers with *Rhizoctonia solani* and *Streptomyces scabies*. *Journal of Plant Nutrition* **28**, 819-833.

Kim H-Y, Choi GJ*, et al.* (2007) Some fungal endophytes from vegetable crops and their anti-oomycete activities against tomato late blight. *Letters in Applied Microbiology* **44**, 332-337.

Kloepper JW (1991) Plant growth-promoting rhizobacteria as biological control agents of soilborne diseases. In 'The biological control of plant diseases'. (Ed. J Bay-Petersen) pp. 142-152. (Food and Fertilizer Technology Center: Taipei, Taiwan)

Konarev AV, Kochetkov VV, Bailey JA, Shrewry PR (1999) The detection of inhibitors of the *Sclerotiorum* (Lib.) de Bary extracellular proteinases in sunflower. *Journal of Phytopathology* **147**, 105-108.

Kulkarni SA, Kulkarni S, Sreenivas MN, Kulkarni S (1997) Interaction between vesicular-arbuscular (VA) mycorrhizae and *Sclerotium rolfsii* Sacc. in groundnut. . *Karnataka Journal of Agricultural Science* **10**, 919-921.

Kwok OCH, Fahy PC, Hoitink HAJ, Kuter GA (1987) Interactions between bacteria and *Trichoderma hamatum* in suppression of *Rhizoctonia* damping-off in bark compost media. *Phylopathology* **77**, 1206-1212.

Larkin RP, Fravel DR (1998) Efficacy of various fungal and bacterial biocontrol organisms for control of fusarium wilt of tomato. *Plant Disease* **82**, 1022-1028.

Larkin RP, Fravel DR (1999) Mechanisms of action and dose-response relationships governing biological control of fusarium wilt of tomato by nonpathogenic *Fusarium* spp. . *Phytopathology* **89**, 1152-1161.

Larkin RP, Hopkins DL, Martin FN (1996) Suppression of Fusarium wilt of watermelon by nonpathogenic *Fusariumm oxysporum* and other microorganisms recovered from a disease suppressive soil. . *Phytopathology* **86**, 812-819.

Latijnhouwers M, de Wit PJGM, Govers F (2003) Oomycetes and fungi: similar weaponry to attack plants. *Trends in Microbiology* **11**, 462-469.

Lazarovits G, Conn KL, Potter J (1999) Reduction of potato scab, verticillium wilt, and nematodes by soymeal and meat and bone meal in two Ontario potato fields. *Canadian Journal of Plant Pathology* **21**, 345-353.

Lee JM (1994) Cultivation of grafted vegetables. I. Current status, grafting methods, and benefits. *HortScience* **29**, 235-239.

Lemanceau P, Bakker PAHM, De Kogel WJ, Alabouvette C, Schippers B (1992) Effect of pseudobactin 358 production by *Pseudomonas putida* WCS358 on suppression of fusarium wilt of carnations by nonpathogenic *Fusarium oxysporum* Fo47. *Applied and Environmental Microbiology* **58**, 2978-2982.

Lemanceau P, Bakker PAHM, Dekogel WJ, Alabouvette C, Schippers B (1993) Antagonistic effect of non-pathogenic *Fusarium oxysporum* Fo47 and pseudobactin 358 upon pathogenic *Fusarium oxysporum* f.sp. dianthi. . *Applied and Environmental Microbiology* **59**, 74-82.

Lifshitz R, Dapler M, Elad Y, Baker R (1984) Hyphal interactions between a mycoparasite, *Pythium nunn* and several soil fungi. *Canadian Journal of Microbiology* **30**, 1482-1487.

Liu L, Kloepper JW, Tuzun S (1995) Induction of systemic resistance in cucumber against fusarium wilt by plant growth promoting rhizobacteria. *Phytopathology* **85**, 695-698.

Loake G, Grant M (2007) Salicylic acid in plant defence – the players and protagonists. *Current Opinion in Plant Biology* **10**, 466-472.

Locke JC, Marois JJ, Papavizas GC (1984) Biological control of Fusarium wilt of greenhouse-grown chrysanthemums. *Plant Disease* **69**, 167-169.

Logsdon G (1993) Using compost for plant disease control. *BioCycle*, 33-36.

Loper JE, Henkels MD (1999) Utilization of heterologous siderophores enhances levels of iron available to *Pseudomonas putida* in the rhizosphere. *Applied and Environmental Microbiology* **63**, 99-105.

Louter HH, Edgington LV (1990) Interaction of cross-protection against Fusarium crown and root rot of tomato. *Canadian Journal of Plant Pathology* **12**, 283-288.

Lovett JV (1982) Agro-chemical alternatives in a future agriculture. *Biological Agriculture and Horticulture* **1**, 15-27.

Lucas GB, Campbell CL, Lucas LT (1992) 'Introduction to plant diseases: Identification and management.' (Kluwer Academic Publishers: Norwell, Massachusetts)

Lumsden RD, Knauss JF (2007) Commercial development of *Trichoderma virens* for damping-off disease. In 'Biological control a global perspective'. (Eds C Vincent, MS Goettel and G Lazarovits) pp. 203-209. (CAB International/AAFC: Oxfordshire, UK)

Lumsden RD, Lewis JA, Millner PD (1982) Composted sludge as a soil amendment for control of soilborne plant diseases. In 'Research for Small Farms'. (Eds HW Kerr Jnr and L Knutson) pp. 274-277. (USDA: Washington DC: GPO)

Lumsden RD, Millner PD, Lewis JA (1986) Suppression of lettuce drop caused by *Sclerotinia minor* with composted sewage sludge. *Plant Disease* **70**, 197-201.

Lyon GD, Reglinski T, Newton AC (1995) Novel disease control compounds: the potential to 'immunize' plants against infection. *Plant Pathology* **44**, 407-427.

Magie RO, Wilfret GJ (1974) Tolerance of *Fusarium oxysporum* f sp. *Gladioli* to benzimidazole fungicides. *Plant Disease Reporter* **58**, 256-259.

Mandavia MK, Kahn NA, Gajera HP, Andharia JH, Parameswaran M (2000) Inhibitory effects of phenolic compounds on fungal metabolism in host-pathogen interactions in *Fusarium* wilt of cumin. . *Allelopathy Journal* **7**, 85-92.

Marois JJ, Locke JC (1985) Population dynamics of *Trichoderma viride* in steamed plant growth medium. *Phytopathology* **75**, 115-118.

Marschner H (1986) 'Mineral nutrition of higher plants.' (Academic press: London)

Marsh AT, Cheah L-H (2001) Crop rotation affects populations of soilborne pathogens in potato soil. *New Zealand Plant Protection* **60**, 310.

Martin FN, Hancock JG (1987) The use of *Pythium oligandrum* for biological control of preemergence damping off caused by *P. ultimum*. *Phytopathology* **77**, 1013-1020.

Matheron M (2001) Modes of action for plant disease management chemistries. In '11th Annual Desert Vegetable Crop Workshop'. (Yuma, Arizona, USA)

Matheron ME, Porchas M (2008) Further evaluation of soil solarization as a management tool for fusarium wilt of lettuce: 2006 field trial. Vegetable Report. The University of Arizona, Yuma Agricultural Center, Yuma.

Mauch-Mani B, Métraux J-P (1998) Salicylic acid and systemic acquired resistance to pathogen attack. *Annals of Botany* **82**, 535-540.

Mayton HS, Oliver C, Vaughn SV, Loria R (1996) Correlation of fungicidal activity of Brassica species with allyl isothiocyanate production in macerated leaf tissue. *Phytopathology* **86**, 267-271.

Mazzola M (1999) Control of replant disease of tree fruits with *Pseudomonas putida*. In. (USA)

Mazzola M (2004) Assessment and management of soil microbial community structure for disease suppression. *Annual Review of Phytopathology* **42**, 35-59.

McConchie R (2007) Activating the plant's natural disease defence system. Melon Industry Report 06/07.

McLean KL, Madsen M, Stewart A (2004) The effect of *Coniothyrium minitans* on sclerotial viability of *Sclerotinia sclerotiorum* and *Ciborinia camelliae*. *New Zealand Plant Protection* **57**, 67-71.

Melzer MS, Boland GJ (1996) Transmissible hypovirulence in *Sclerotinia minor*. *Canadian Journal of Plant Pathology* **18**, 19-28.

Metcalf D, Groom T, Van Essen A, Kitchener T (2004) *Trichoderma* - a predator of onion white rot. *Onions Australia* **21**, 27-28.

Milgroom MG, Cortesi P (2004) Biological control of a chestnut blight with hypovirulence: A critical analysis. *Annual Review of Phytopathology* **42**, 311-338.

Miyaki Y, Takahashi E (1983) Effect of silicon on the growth of solution-cultured cucumber plants. *Japanese Journal of Soil Science and Plant Nutrition* **29**, 71-83.

Momma N (2008) Review - Biological soil disinfestation (BSD) of soilborne pathogens and its possible mechanisms. *Japan Agricultura Research Quarterly* **42**, 7-12.

Monteiro-Vitorello CB, Bell JA, Fulbright DW, Bertrand H (1995) A cytoplasmically transmissible hypovirulence phenotype associated with mitochondrial DNA mutations in the chestnut blight fungus *Cryphonectria parasitica*. *Proceedings of the National Academy of Science USA* **92**, 5935-5939.

Montesinos E, Bonaterra A, Badosa E, Francés J, Alemany J, Llorente I, Moragrega C (2002) Plant-microbe interactions and the new biotechnological methods of plant disease control. *International Microbiology* **5**, 169-175.

Moorman GW, Arora N, Kim SH (2002a) Propamocarb resistance in species of *Pythium*. *Phytopathology* **92**, S58 (meeting abstract).

Moorman GW, Kang S, Geiser DM, Kim SH (2002b) Identification and characterisation of *Pythium* species associated with greenhouse floral crops in Pennsylvania. *Plant Disease* **86**, 1227-1231.

Moorman GW, Kim SH (2004) Species of *Pythium* from greenhouses in Pennsylvania exhibit resistance to propamocarb and mefenoxam. *Plant Disease* **88**, 630-632.

Mou Z, Fan W, Dong X (2003) Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. *Cell* **113**, 935-944.

Murakami H, Tsushima S, Akimoto T, Kanno T, Shishido Y (2003) Effects of cultivation of preceding plants on the development of fusarium wilt of cucumber and daikon. *Soil Science and Plant Nutrition* **49**, 703-710.

Murakami H, Tsushima S, Kuroyanagi Y, Shishido Y (2002) Reduction of resting spore density of *Plasmodiophora brassicae* and clubroot disease severity by liming. *Soil Science and Plant Nutrition* **48**, 685-691.

Myers DF, Campbell RN (1985) Lime and the control of clubroot of crucifers: effects of pH, calcium, magnesium, and their interactions. *Phytopathology* **75**, 670-673.

Myers DF, Campbell RN, Greathead AS (1981) Clubroot of brassicas in California: Soils respond differently to lime for clubroot control. *Phytopathology* **71**, 1005-1006.

Nakasaki K, Hiraoka S, Nagata H (1998) A new operation for producing disease-suppressive compost from grass clippings. *Applied and Environmental Microbiology* **64**, 4015-4020.

Naseby DC, Pascual JA, Lynch JM (2000) Effect of biocontrol strains of *Trichoderma* on plant growth, *Pythium ultimum* populations, soil micorbial communities and soil enzyme activities. *Journal of Applied Microbiology* **88**, 161-169.

Nelson EB, Burpee LL, Lawton MB (1994) Biological control of turfgrass diseases. In 'Handbook of Integrated Pest Management for Turf and Ornamentals'. (Ed. AR Leslie) pp. 409-427. (Lewis Publishers: Ann Arbor, MI)

Nicholson RL (1992) Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology* **30**, 369-389.

Nigro F, Finetti Sialer MM, Gallitelli D (1999) Transformation of *Metschnikowia pulcherrima* 320, a biocontrol agent of storage rot, with the green fluorescent protein gene. *Journal of Plant Pathology* **81**, 205-208.

Nuss DL, Koltin Y (1990) Significance of dsRNA genetic elements in plant pathogenic fungi. *Annual Review of Phytopathology* **28**, 37-58.

O'Brien DG, Dennis RWG (1936) Further information relating to control of raan in swedes. *Scottish Journal of Agriculture* **19**, 1-18.

O'Callaghan M, Swaminathan J, Lottmann J, Wright DA, Jackson TA (2006) Seed coating with biocontrol strain *Pseudomonas fluorescens* F113. *New Zealand Plant Protection* **59,** 80-84.

Olaya G, Cleere S, Stanger C, Burbidge J, Hall A, Windass J (2003) A novel potential target site of QoI fungicide resistance mechanism in *Pythium aphanidermatum*. *Phytopathology* **93**, S67.

Oliver RP, Ipcho SVS (2004) *Arabidopsis* pathology breathes new life into the necrotrophs-vs.-biotrophs classification of fungal pathogens. *Molecular Plant Pathology* **5**, 347-352.

Ordentlich A, Elad Y, Chet I (1988) The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. *Phytopathology* **78**, 84-88.

Ozbay N, Newman SE, Brown WM (2004) Evaluation of *Trichoderma harzianum* strains to control crown and root rot of greenhouse fresh market tomatoes. *Acta Horticulturae* **635**.

Ozores-Hampton M, Obreza TA, Hochmuth G (1998) Using composted wastes on florida vegetable crops. *HortTechnology* **8**, 130-137.

Ozores-Hampton MP, Obreza TA (1998) Use of composts on Florida's vegetable crops. In 'Compost Use in Florida'. (Ed. FCfSaHwmftFDoE Protection) pp. 39-42: Florida)

Papavizas GC (1985) *Trichoderma* and *Gliocladium*: Biology, ecology and potential for biocontrol. *Annual Review of Phytopathology* **23**, 23-54.

Parbery DG (1996) Trophism and the ecology of fungi associated with plants. *Biological Reviews* **71**, 473-527.

Parke JL, Gurian-Sherman D (2001) Diversity of the *Burkholderia cepacia* complex and implications for risk assessment of biological control strains. *Annual Review of Phytopathology* **39**, 225-258.

Paroussi G, Bletsos F, Bardas GA, Kouvelos JA, Klonari A (2007) Control of *fusarium* and *verticillium* wilt of watermelon by grafting and its effect on fruit yield and quality. *Acta Horticulturae* **729**, 281-285.

Pascual JA, Garcia C, Hernandez T, Lerma S, Lynch JM (2002) Effectiveness of municipal waste compost and its humic fraction in suppressing *Pythium ultimum*. *Microbial Ecology* **44**, 59-68.

Patterson CL, Grogan RG (1985) Differences in epidemiology and control of lettuce drop caused by *Sclerotinia minor* and *S. sclerotiorum*. *Plant Disease* **69**, 766-770.

Pavlou GC, Vakalounakis DJ, Ligoxigakis EK (2002) Control of root and stem rot of cucumber, caused by *Fusarium oxysporum* f. sp. *Radicis-cucumerinum*, by grafting onto resistant rootstocks. *Plant Disease* **86**, 379-382.

Peniston QP, Johnson EL (1980) Process for the manufacture of chitosan. Patent number 4195175. 5pp.

Perez C, Dill-Macky R, Kinkel LL (2008) Management of soil microbial communities to enhance populations of *Fusarium graminearum*-antagonists in soil. *Plant and Soil* **302**, 53-69.

Perfect ES, Green JR (2001) Infection structures of biotrophic and hemibiotrophic fungal plant pathogens. *Molecular Plant Pathology* **2**, 101-108.

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

Pleban S, Chernin L, Chet I (1997) Chitinolytic activity of an endophytic strain of *Bacillus cereus*. *Letters in Applied Microbiology* **25**, 284-288.

Porter IJ (1991) Factors which influence the effectiveness of solarization for control of soilborne fungal pathogens in South Eastern Australia. PhD thesis. La Trobe University.

Porter IJ, Merriman PR (1983) Effects of solarization of soil on nematode and fungal pathogens at two sites in Victoria. *Soil Biology and Biochemistry* **15**, 39-44.

Prabhu AS, Fageria NK, Berni RF, Rodrigues FÁ (2007a) Phosphorus and plant disease. In 'Mineral nutrition and plant disease'. (Eds LE Datnoff, WH Elmer and DM Huber) pp. 45-56. (The American Phytopathological Society: St. Paul, Minnesota)

Prabhu AS, Fageria NK, Huber DM, Rodrigues FÁ (2007b) Potassium and plant disease. In 'Mineral nutrition and plant disease'. (Eds LE Datnoff, WH Elmer and DM Huber) pp. 57-78. (The American Phytopathological Society: St Paul, Minnesota)

Pryor B (2007) Evaluation of *Paenibacillus polymyxa* as a biocontrol agent against *Sclerotinia minor* in Lettuce. Report for the California Lettuce Research Board. University of Arizona.

Pullman GS, DeVay JE, Garber RH (1981) Soil Solarization and Thermal Death: A Logarithmic Relationship Between Time and Temperature for Four Soilborne Plant Pathogens. *Phytopathology* **71**, 959-964.

Pullman GS, DeVay JE, Garber RH, Weinhold AR (1979) Control of soil-borne fungal pathogens by plastic tarping of soil. In 'Soil-borne Plant Pathogens'. (Eds B Schippers and W Gams) pp. 439-446. (Academic Press: London)

Pung H (2007) New fungicides and strategies for sustainable management of *Sclerotinia* and *Rhizoctonia* diseases on vegetable crops in Australia. Milestone report. Peracto Pty Ltd.

Pung H, Villalta O, Cross S, Wilson D, Porter IJ (2005) Alternative fungicides to procymidone for Sclerotinia disease control on horticultural crops. In '15th Biennial Australasian Plant Pathology Society Conference'. Geelong, Victoria p. 323

Punja ZK, Carter JD, Campbell GM, Rossell EL (1986) Effects of calcium and nitrogen fertilizers, fungicides, and tillage practices on incidence of *Sclerotium rolfsii* on processing carrots. *Plant Disease* **70**, 819-824.

Punja ZK, Utkhede RS (2003) Using fungi and yeasts to manage vegetable crop diseases. *Trends in Biotechnology* **21**, 400-407.

Raaijmakers JM, Leeman M, Van Oorschot MMP, Van der Sluis I, Schippers B, Bakker PAHM (1995) Dose-response relationships in biological control of fusarium wilt of radish by *Pseudomonas* spp. *Phytopathology* **85**, 1075-1081.

Rahman M, Punja ZK (2007) Calcium and plant disease. In 'Mineral nutrition and plant disease'. (Eds LE Datnoff, WH Elmer and DM Huber) pp. 79-93. (The American Phytopathological Society: St Paul, Minnesota)

Ramirez-Villapudua J, Munnecke DE (1988) Effect of solar heating and soil amendments of cruciferous residues on *Fusarium oxysporum* f. sp. *conglutinans* and other organisms. *Phytopathology* **78**, 289-295.

Raviv M (2008) The use of compost in growing media as suppressive agent against soilborne diseases. *Acta Horticulturae* **779**, 39-50.

Reglinski T, Whitaker G, Cooney JM, Taylor JT, Poole PR, Roberts PB, Kim KK (2001) Systemic acquired resistance to *Sclerotinia sclerotiorum* in Kiwifruit vines. *Physiological and Molecular Plant Pathology* **58**, 111-118.

Reuveni R, Dudai N, Putievski E (1998) Nufar: a sweet basil cultivar resistant to Fusarium wilt. *HortScience* **33**, 159.

Ridgway HJ, Rabeendran N, Eade K, Stewart A (2001) Application timing of *Coniothyrium minitans* A69 influences biocontrol of *Sclerotinia minor* in lettuce. *New Zealand Plant Protection* **54**, 89-92.

Ringer CE, Millner PD, Teerlinck LM, Lyman BW (1997) Suppression of seedling damping-off disease in potting mix containing animal manure composts. *Compost Science and Utilization* **5**, 6-14.

Roberts W, Bruton BD, Fish WW, Taylor MJ (2007) Using grafted transplants in watermelon production. In '2007 Southeast Regional Vegetable Conference'. Savannah, Georgia, USA. (Ed. WT Kelly) pp. 33-36

Rodrigues FA, Datnoff LE (2005) Silicon and rice disease management. *Fitopatologia Brasileira* **30**, 457-469.

Rodrigues FÁ, Silva OA, Juliatti FC, Korndörfer GH, Corrêa GF, Peixoto JR (1996) Effect of calcium silicate on the development of *Fusarium oxysporum* f. sp. *lycopersici,* races 1 and 2, on tomato. *Fitopatologia Brasileira* **21 (suppl.)**, 393.

Roe NE, Stoffella PJ, Bryan HH (1994) Growth and yields of bell pepper and winter squash grown with organic and living mulches. *Journal of the American Society for Horticultural Science* **119**, 1193-1199.

Ross AF (1961) Systemic acquired resistance induced by localized virus infections in plants. *Virology* **14**, 340-358.

Ryals J, Uknes S, Ward E (1994) Systemic acquired resistance. *Plant Physiology* **104**, 1109-1112.

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner H-Y, Hunt MD (1996) Systemic acquired resistance. *Plant Cell* **8**, 1809-1819.

Savard ME, Melzer MS, Boland GJ, Bensimon C, Blackwell BA (2003) A new 1-hydroxy-2,6-pyrazinedione asociated with hypovirulent isolates of *Sclerotinia minor*. *Journal of Natural Products* **66**, 306-309.

Schoonbeek H-J, Jacquat-Bovet A-C, Mascher F, Métraux JP (2007) Oxalate degrading bacteria can protect Arabidopsis thaliana and crop plants against Botrytis cinerea. *Molecular Plant Microbe Interactions* **20(12)**, 1535-1544.

Sekiguchi A (1977) Control of Fusarium wilt on Chinese yam. *Annual Report of the Department of Plant Pathology, Entomology, Vegetables and Floriculture Experimental Station Nagano Japan* **1**, 10-11.

Sekine T, Sugano M, Majid A, Fujii Y (2007) Antifungal effects of volatile compounds from Black Zira (*Bunium persicum*) and other spices and herbs. *Journal of Chemical Ecology* **33**, 2123-2132.

Sekizawa Y, Mase S (1981) Mode of controlling action of probenazole against rice blast disease with reference to the induced resistance mechanism in rice plant. *Journal of Pesticide Science* **6**, 91-94.

Shim M-Y, Starr JL, Neller KP, Woodard KE, Lee TAJ (1998) Distribution of isolates of *Sclerotium rolfsii* tolerant to pentachloronitrobenzene in Texas peanut fields. *Plant Disease* **82**, 103-106.

Singh PP, Shin YC, Park CS, Chung YR (1999) Biological control of *Fusarium* wilt of cucumber by chitinolytic bacteria. *Phytopathology* **89**, 92-99.

Singh S, Chand H (2006) Screening of bioagents against root rot of mung bean caused by *Rhizoctonia solani*. *Communications in Applied Biological Sciences* **71**, 33-35.

Sivan A, Chet I (1986) Possible mechanisms of control of *Fusarium* spp. by *Trichoderma harzianum*. In 'British Crop Protection Conference Pests and Diseases'. Brighton Metropole, England pp. 865-872. (BCPC)

Smith BJ, Kirkegaard JA (2002) In vitro inhibition of soil microorganisms by 2-phenylethyl isothiocyanate. *Plant Pathology* **51**, 585-539.

Smith FD, Phipps PM, Stipes RJ, Brenneman TB (1995) Significance of insensitivity of *Sclerotinia minor* to iprodione in control of Sclerotinia blight of peanut. *Plant Disease* **79**, 517-523.

Smolinska U, Horbowicz M (1999) Fungicidal activity of volatiles from selected cruciferous plants against resting propagules of soil-borne fungal pathogens. *Journal of Phytopathology* **147**, 119-124.

Sneh B (1998) Use of non-pathogenic or hypovirulent fungal strains to protect plants against closely related fungal pathogens. *Biotechnology Advances* **16**, 1-32.

Sneh B, Henis Y (1972) Production of antifungal substances active against *Rhizoctonia solani* in chitin-amended soil. *Phytopathology* **62**, 595-600.

Sneh B, Ichielevich-Auster M (1998) Induced resistance of cucumber seedlings caused by some non-pathogenic *Rhizoctonia* (np-R) isolates. *Phytoparasitica* **26**, 27-38.

Sneh B, Ichielevich-Auster M, Schomer I (1989) Mechanism of seedling protection induced by a hypovirulent isolate of *Rhizoctonia solani*. *Canadian Journal of Plant Pathology* **67**, 2135-2141.

Sneh B, Yamoah E, Stewart A (2004) Hypovirulent *Rhizoctonia* spp. isolates from New Zealand soils protect radish seedlings against damping-off caused by *R. solani*. *New Zealand Plant Protection* **57**, 54-58.

Soylu S, Yigitbas H, Soylu EM, Kurt Ş (2007) Antifungal effects of essential oils from oregano and fennel on *Sclerotinia sclerotiorum*. *Journal of Applied Microbiology*.

Sreeramulu KR, Onkarappa T, Swamy HN (1998) Biocontrol of damping off and black shank disease in tobacco nursery. *Tobacco Research* **24**, 1-4.

Stanghellini ME, Kim DH, Rasmussen SL, Rorabaugh PA (1996a) Control of root rot of peppers caused by *Phytophthora capsici* with a nonionic surfactant. *Plant Disease* **80**, 1113-1116.

Stanghellini ME, Miller RM (1997) Biosurfactants their identity and potential efficacy in the biological control of zoosporic plant pathogens. *Plant Disease* **81**, 4-12.

Stanghellini ME, Nielsen CJ, Kim DH, Rasmussen SL, Rorabaugh PA (2000) Influence of sub- versus top-irrigation and surfactants in a recirculating system on disease incidence caused by *Phytophthora* spp. in potted pepper plants. *Plant Disease* **84**, 1147-1150.

Stanghellini ME, Rasmussen SL, Kim DH, Rorabaugh PA (1996b) Efficacy of nonionic surfactants in the control of zoospore spread of *Pythium aphanidermatum* in a recirculating hydroponic system. *Plant Disease* **80**, 422-428.

Stanghellini ME, Tomlinson JA (1987) Inhibitory and lytic effects of a non-ionic surfactant on various asexual stages in the life cycle of *Pythium* and *Phytophthora* species. *Phytopathology* **77**, 112-114.

Stangoulis JCR, Graham RD (2007) Boron and plant disease. In 'Mineral nutrition and plant disease'. (Eds LE Datnoff, WH Elmer and DM Huber) pp. 207-214. (The American Phytopathological Society: St Paul, Minnesota)

Sticher L, Mauch-Mani B, Métraux JP (1997) Systemic acquired resistance. *Annual review of Phytopathology* **35**, 235-270.

Stigter HCM (1971) Some aspects of the physiological functioning of the graft muskmelon/*Cucurbita ficifolia*. *Pflanzenphysiologie* **65**, 223-231.

Stirling GR (2008) The impact of farming systems on soil biology and soilborne diseases: examples from the Australian sugar and vegetable industries - the case for better integration of sugarcane and vegetable production and implications for future research. *Australasian Plant Pathology* **37**, 1-18.

Stirling GR, Eden LM (2008) The impact of organic amendments, mulching and tillage on plant nutrition, Pythium root rot, root-knot nematode and other pests and diseases of capsicum in a subtropical environment, and implications for the development of more sustainable vegetable farming systems. *Australasian Plant Pathology* **37**, 123-131.

Stoffella PJ, Li Y, Roe NE, Ozores-Hampton M, Graetz DA (1996) Utilization of organic waste composts in vegetable crop production systems. In 'Managing soil fertility for intensive vegetable production systems in Asia'. (Ed. RA Morris) pp. 252-269. (Asian Vegetable Research and Development Centre: Tiawan)

Stovold G (2006) Using fungicides correctly. Spray Sense, information on pesticide issues. (Ed. 7). NSW Department of Primary Industries.

Sturz AV (2006) Bacterial root zone communities, beneficial allelopathies and plant disease control. In 'Allelochemicals: biological control of plant pathogens and diseases'. (Eds Inderjit and KG Mukerji) pp. 214. (Springer: Dordrecht, The Netherlands)

Subbarao KV (2004) Impact of crop residue amendments on soilborne disease management. In 'Claifornia Conference on Biological Control IV Berkeley, California, USA'. Centre for Biological Control, College of Natural Resources, University of California, Berkeley, California, USA pp. 13-15

Sullivan P (2004) Sustainable management of soilborne plant diseases. **IP173**. ATTRA National Sustainable Agriculture Information Service, California.

Tamietti G, Valentino D (2006) Soil solarization as an ecological method for the control of *Fusarium* wilt of melon in Italy. *Crop Protection* **25**, 389-397.

Tenuta M, Conn KL, Lazarovits G (2002) Volatile fatty acids in liquid swine manure can kill microsclerotia of *Verticillium dahliae*. *Phytopathology* **92**, 548-552.

Tenuta M, Lazarovits G (2002) Ammonia and nitrous acid from nitrogenous amendments kill microsclerotia of *Verticillium dahliae*. *Phytopathology* **92**, 255-264.

Thaler JS, Owen B, Higgins VJ (2004) The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. *Plant Physiology* **135**, 530-538.

Thanassoulopoulos CC, Giannopilitis CN, Kitsos GT (1970) Evaluation of sensitiveness and development of resistance of *Fusarium oxysporum* f.sp. *lycopersici* to benomyl. *Phytopathologischea Zeitschrift* **70**, 114-120.

Thomma B, Tierens KFM, Penninckx I, Mauch-Mani B, Broekaert WF, Cammue BPA (2001) Different micro-organisms differentially induce *Arabidopsis* disease response pathways. *Plant Physiology and Biochemistry* **39**, 673-680.

Thomma BPHJ, Eggermont K, Penninckx IAMA, Mauch-Mani B, Vogelsang R, Cammue BPA, Broekaert WF (1998) Separate jasmonate-dependant and salicylate-dependant defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proceedings of the National Academy of Science USA* **95**, 15107-15111.

Tikoo SK, Mathai PJ, Kishan R (1979) Successful graft culture of tomato in bacterial wilt sick soil. *Current Science* **48**, 259-260.

Tilston EL, Pitt D, Groenhof AC (2002) Composted recycled organic matter suppresses soil-borne diseases of field crops. *New Phytologist* **154**, 731-740.

Tivoli B, Deltour A, Molet D, Bedin P, Jouan B (1986) Isolation of thiabendazole resistant strains of *Fusarium roseum* var *sambucinum* from potato tubers. *Agronomie* **6**, 219-224.

Tomio K, Takio I (1998) Hyphal interactions between *Pythium ultimum* and a mycoparasite, *P. oligandrum* - Light microscopic observations of their interface in and around cucumber roots in soil. *Nippon Kin Gakkai Kaiho* **39**, 159-168.

Tomlinson JA, Faithfull EM (1979) Effects of fungicides and surfactants on the zoospores of *Olpidium brassicae*. *Annals of Applied Biology* **93**, 13-19.

Tomlinson JA, Faithfull EM (1980) Studies on the control of lettuce big-vein disease in recirculated nutrient solutions. *Acta Horticulturae* **98**, 325-331.

Tomlinson JA, Thomas BJ (1986) Studies on melon necrotic spot virus disease of cucumber and on the control of the fungus vector (*Olpidium radicale*) *Annals of Applied Biology* **108**, 71-80.

Toyoda H, Hashimoto H, Utsumi R, Kobayashi H, Ouchi S (1988) Detoxification of fusaric acid by a fusaric acid-resistant mutant of *Pseudomonas solanacearum* and its application to biological control of fusarium wilt of tomato. *Phytopathology* **78**, 1307-1311.

Toyoda H, Utsumi R (1991) Method for the prevention of *Fusarium* diseases and microorganisms used for the same. In. (USA)

Tribe HT (1957) On the parasitism of *Sclerotinia trifoliorum* by *Coniothyrium minitans*. *Transactions of the British Mycological Society* **40**, 489-499.

Tu JC (1985) Tolerance of white bean (*Phaseolus vulgaris*) to white mould (*Sclerotinia sclerotiorum*) associated with tolerance to oxalic acid. *Physiol. Plant Pathology* **26**, 111-117.

Valaskova E (1983) The influence of various carbon and nitrogen sources on mycelium growth and resistance stability in benomyl tolerant strain of *Fusarium oxysporum* f sp. *Tulipae*. *Sbornik UVTIZ (Ustav Vedeckotechnickych Informaci pro Zemedelstvi) Ochrana Rostlin* **19**, 251-260.

Vallad GE, Goodman RM (2004) Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science* **44**, 1920-1934.

van der Gaag DJ, van Noort FR, Stapel-Cuijpers LHM, de Kreij C, Termorshuizen AJ, van Rijn E, Zmora-Nahum S, Chen Y (2007) The use of green waste compost in peat-based potting mixtures: Fertilization and suppressiveness against soilborne diseases. *Scientia Horticulturae* **114**, 289-297.

van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology* **36**, 453-483.

van Peer R, Schippers B (1992) Lipopolysaccharides of plant-growth promoting *Pseudomonas* sp. strain WCS417r induce resistance in carnation to fusarium wilt. *Netherlands Journal of Plant Pathology* **98**, 129-139.

Vesely D, Hejdnek S (1984) Microbial relations of *Pythium oligandrum* and problems in the use of this organism for the biological control of damping off in sugar beet. *Zbl Microbiol* **139**, 257-265.

Vijayan P, Shockey J, Levesque CA, Cook RJ, Browse J (1998) A role for jasmonate in pathogen defense of *Arabidopsis*. *Proceedings of the National Academy of Science USA* **95**, 7209-7214.

Villajuan-Abgona R, Kageyama K, Hyakumachi M (1996) Biological control of Rhizoctonia damping off of cucumber by non-pathogenic binucleate *Rhizoctonia*. *European Journal of Plant Pathology* **102**, 227-235.

Villalta O, Porter IJ*, et al.* (2004) Development of biological controls for *Sclerotinia* diseases of horticultural crops. Horticulture Australia Ltd.

Villalta O, Pung H (2004) Screening of bean cultivars (*Phaseolus vulgaris*) for resistance to *Sclerotinia sclerotiorum* at field sites in Victoria and Tasmania, Australia. Industry report. Department of Primary Industries, Knoxfield.

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M (2008) *Trichoderma*-plant-pathogen interactions. *Soil Biology and Biochemistry* **40**, 1-10.

Voland RP, Epstein AH (1994) Development of suppressiveness to diseases caused by *Rhizoctonia solani* in soils amended with composted and noncomposted manure. *Plant Disease* **78**, 461-466.

Walker JC (1946) Soil management and plant nutrition in relation to disease development. *Soil Science* **61**, 47-54.

Walker JC, Morrel S, Foster HH (1937) Toxicity of mustard oils and related sulphur compounds to certain fungi. *American Journal of Botany* **24**, 536-541.

Walker R, Morris S, Brown P, Gracie A (2004) Evaluation of potential for chitosan to enhance plant defence. Rural Industries Research and Development Corporation.

Wang Y, Fristensky B (2001) Transgenic canola lines expressing pea defence gene DRR206 have resistance to aggressive blackleg isolates and to *Rhizoctonia solani*. *Molecular Breeding* **8**, 263-271.

Webster MA, Dixon GR (1991a) Boron, pH and inoculum concentration influencing colonization by Plasmodiophora brassicae. *Mycological Research* **95**, 74-79.

Webster MA, Dixon GR (1991b) Calcium, pH and inoculum concentration influencing colonization by *Plasmodiophora brassicae*. *Mycological Research* **95**, 65-73.

Welch N, Greathead AS, Inman J, Quick J (1976) Clubroot control in Brussels sprouts using lime for pH adjustment. *California Agriculture*, 10-11.

Weller DM, Raaijmakers JM, McSpadden Gardner BB, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology* **40**, 309-348.

Whan JH (1976) Tolerance of *Sclerotinia fructicola* to benomyl. *Plant Disease Reporter* **60**, 200-201.

Whipps JM, Gerlagh M (1992) Biology of *Coniothyrium minitans* and its potential in disease control. *Mycological Research* **96**, 897-907.

White GA, Stanghellini ME, Ayoubi LM (1988) Variation in the sensitivity to metalaxyl of *Pythium* spp isolated from carrot and other sources. *Annals of Applied Biology* **113**, 269-278.

Wicks T, Horsman C, Hall B (2001) Report to Australian Agricultural Marketing Organisation on the effects of volatiles from PERLKA on the growth of selected fungal pathogens of potato and carrot. South Australian Research and Development Institute.

Wong SP (2003) Past, current and future challenges for fungicide resistance management of turfgrass pathogens. *Phytopathology* **93 supplement 6**, S114.

Wright Dea (2003) Improvement in lettuce quality by reduction in losses due to soil borne diseases. Horticulture Australia Limited.

Wright PJ (2007) Control of Sclerotinia leaf drop of lettuce. In '16th Biennial Australasian Plant Pathology Society Conference'. Adelaide, South Australia p. 121

Xavier LJC, Boyetchko SM (2002) Arbuscular mycorrhizal fungi as biostimulants and bioprotectants of crops. *Applied Mycology and Biotechnology* **2**, 311-339.

Xu Y, Chang P-F, Liu D, Narasimhan ML, Raghothama KG, Hasegawa PM, Bressan RA (1994) Plant defense genes are synergistically induced by ethylene and methyl jasmonate. *Plant Cell* **6**, 1077-1085.

Yang R, Han YC, Li GQ, Jiang DH, Huang HC (2007) Suppression of *Sclerotinia sclerotiorum* by antifungal substances produced by the mycoparasite *Coniothyrium minitans*. *European Journal of Plant Pathology* **119**, 411–420.

Yasuda M, Kusajima M, Nakajima M, Akutsu K, Kudo T, Yoshida S, Nakashita H (2006) Thiadiazole carboxylic acid moiety of tiadinil, SV-03, induces systemic acquired resistance in tobacco without salicylic acid accumulation. *Journal of Pesticide Science* **31**, 329-334.

Yasuda M, Nakashita H, Yoshida S (2004) Tiadinil, a novel class of activator of systemic acquired resistance, induces defense gene expression and disease resistance in tobacco. *Journal of Pesticide Science* **29**, 46-49.

Zavaleta-Mejia E, Rojas RIM (1990) The effects of Brassica crop residues on *Sclerotium cepivorum* Berk. . In '4th International Workshop on Allium White Rot'. Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig, Germany. (Eds AR Entwiste and P Mattusch) pp. 185-195

Zeng R-S (2006) Disease resistance in plants through mycorrhizal fungi induced allelochemicals. In 'Allelochemicals: biological control of plant pathogens and diseases'. (Eds Inderjit and KG Mukerji) pp. 181-192. (Springer: Dordrecht, The Netherlands)

Zhang S, Schisler DA, Boehm MJ, Slininger PJ (2007) Utilization of chemical inducers of resistance and *Cryptococcus flavenscens* OH 182.9 to reduce Fusarium head blight under greenhouse conditions. *Biological Control* **42**, 308-315.

Zhang W, Dick WA, Hoitink HAJ (1996) Compost induced systemic acquired resistance in cucumber to *Pythium*, root rot and anthracnose. *Phytopathology* **83**, 1066-1070.

Zhou T, Boland GJ (1997) Hypovirulence and double stranded RNA in *Sclerotinia homoeocarpa*. *Phytopathology* **87**, 147-153.

Zhou T, Boland GJ (1998a) Biological control strategies for *Sclerotinia species*. In 'Plant-microbe interactions and biological control'. (Eds GJ Boland and LD Kuykendall) pp. 127-156. (Marcel Dekker, Inc.: New York)

Zhou T, Boland GJ (1998b) Suppression of dollar spot by hypovirulent isolates of *Sclerotinia homoeocarpa*. *Phytopathology* **88**, 788-794.

Zhou T, Boland GJ (1999) Mycelial growth and production of oxalic acid by virulent and hypovirulent isolates of *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology* **21**, 93-99.

Zhou T, Paulitz TC (1994) Induced resistance in the biocontrol of *Pythium aphanidermatum* by *Pseudomonas* spp. on cucumber. *Journal of Phytopathology* **142**, 51-63.

Zitter TA (1998) Fusarium diseases of cucurbits. Vegetable MD Online. Cornell University, Ithaca, NY.

1.19 Appendix 1.1 – IPM based research priorities identified at pathogen specific workshops

Workshops were conducted at the beginning of the project - Rhizoctonia (16-17 Oct 2007, Victoria), Pythium & Fusarium (23 Nov 2007, New South Wales) and Sclerotina (28-29 Nov 2007, Tasmania). A full participants list is included in the acknowledgements (1.17).

1.19.1 Sclerotinia

Sclerotinia minor – Lettuce (in priority order)	*Sclerotinia sclerotiorum* – Beans (** denotes highest priority)

1. Fungicide protection
• Reregister procymidone
• More chemical groups and modes of action (downward movement but better than phos acid, investigate products in IR4 program, Consider fluazinam, (Shirlan) +/- wetter)
• Information on methods to reduce spray number
• New safe systemic fungicide required.
• Improve application methods for existing fungicides
• Develop resistance management guidelines
• Concern over persistence of Filan in soils
 	
2. **Inoculum reduction methods needing consideration:**
• Alternative biocides – softer
• Plant extracts – oils (eg rosemary, thyme oil, garlic, cloves, Solenium, sulphur)
• Antagonistic crops
• Germination stimulants (identify germination mechanisms for *S. minor*)
• Alternative oil products (cu octenoe)
• Volatiles from organic matter
• Degrading products from insects/animals
 	
3. Direct treatment of sclerotia on crop residues:
• Application of control methods (eg. fungicides) during growth or post harvest
• After harvest of a crop spray herbicide or Contans™ to kill surface sclerotia carrying over into next crop
• Apply lime post harvest
• Treat infected areas only – chipping out infected plants when weeding – would work well for fields with less than 5 % infection?
• Sclerotial degrader (skin bleaches etc)
• Melanin disrupters
• Fungal gnats and Nematodes
 	
4. Plant resistance: (Mechanisms to consider)
• Antifungal proteinases
• Oxalate oxidase
• Screen resistant plants from the USA.
• Consider the use of GM mechanisms and
	1. Inoculum reduction
	• Fumigants, especially consider DMDS
	• Biofumigant crops and soil amendments
	• Controlled traffic
	• **Preplant soil treatments (chemical, biofumigant crops, biocontrol agents eg. Contans™)
	• Soil organisms to eat away sclerotia (only slugs and snails known so far, no good as these are pests)
	
	2. Fungicide application
	• Optimise fungicide control
	• Timing of application vs open flowers and whether chemicals can transmit through flower sheath
	• Application method (nozzle, air accelerated)
	• Raise pH in fungicide spray to above pH 7 (to reduce optimum conditions for invasion of plant tissue)
	• ** Fungicide resistance management
	  ○ Limited products
	  ○ 'Banrod' used in US
	  ○ Sumisclex® (procymidone)
	  ○ Chemical use patterns – ie. Restrict use of when you can apply procymidone.
	• Surfactant effects
	
	3. Spread of infection
	• Ascospore detection, predicting release
	• Inhibiton of apothecia with chemicals/herbicides
	• Calcium hydroxide or similar for pH adjustment, soil surface application to inhibit apothecial production
	• Wheat straw or mulch to prevent apothecial production – if mulch thick enough this may prevent spore release. It may also have additional benefits
	
	4. Reducing disease and crop conditions (crop conditions that favour disease)
	• Row orientation air flow
	• Varieties
	• Crop canopy
	• Irrigation manipulation – trials/drippers
	• Fertiliser

crops **5. Surface soil modification (Barriers)** • Nutrient eg. CaO, Perlka® • Biological • Physical (expensive) • Stay on films • Erosion control products **6. Improve general soil health and look for inoculum suppression**	• Healthy root system, reduce frequency of irrigation • Flowering period – shorten **5. Other** • Petal/plant pH vs susceptibility of infection • Biocontrol – Contans® used in USA – Used as foliar spray on flowers • **Better understanding of severe infected crops – condition of crops, field etc

1.19.2 Rhizoctonia (vegetable and potatoes)

1. Improve understanding of Rhizoctonia
Particularly in vegetable crops
Research – epidemiology, identify anastomisis groups (AG), pathogenicity, infection sites, AGs
Epidemiology – what allows Rhizoctonia to become pathogenic
Chemical management – targeting different AG groups – different active ingredients

2. Enhance understanding of disease suppression
(soil health and biological)
Method of microbial profiling which identifies a reduction in rhizoctonia disease and inoculum
Development of suppressive soil conditions

3. Determine benefit/impact of crop rotation on disease
Paddock selection
Benchmark diagnostics – sampling, crop loss/damage
Vegetables – desk top study crop rotations that reduce inoculum, management practices
Understand how different crops influence soil inoculum loads

4. Conduct knowledge transfer
Web based database with grower inputted data, defined end use, managing group
Best practice manual (revised after Peru)
Deliver information on: R&D short-medium, inoculum, seed, soil vs disease and soil vs damage/yield, crop rotation, seed health, chemical, cultural, biologicals, diagnostics, disease prediction, epidemiology
Potato knowledge transfer
Super trials
To include ideas and solutions, field sites, farmer trials and talks

5. Understand impact of seed inoculum
Seed vs soil-borne on different AG groups
Impact of storage on inoculum levels
Certification control
Threshold for clean seed
Infection point
No certification control
Certified seed growers
Thresholds on DNA test
Cost benefit analysis of rhizoctonia free seed

6. Determine impact of nutrients on disease
(Crop management and cultural control)
Best practice nutrient controls (controlled environment studies) (eg. Calcium nitrate and exudates)
Cultural controls (eg. Nutrients, amendments and biologicals)

1.19.3 Fusarium and Pythium

Fusarium (field)	**Fusarium (greenhouse)**	**Pythium (field)**	**Pythium (greenhouse)**
1. Resistance (if available) Breeding, SAR, grafting 2. Inoculum reduction Systemic fungicides, crop residue management, rotation, novel fumigation strategies, organic amendments, non-pathogenic fusariums/biologicals 3. Farm Hygiene Extension, economic analysis 4. Environment Timing of sowing	1. New chemistry including application techniques and strategic application 2. Biological control Identify effective biocontrol agents 3. SAR and other suppressive media (eg. composts) 4. Irrigation management 5. Resistant cultivars 6. Diagnosis (are there Australian strains?) 7. Extension (a good DVD on management strategies)	1. Irrigation management (need data and advice on sandy vs clay soils) 2. Use of phosphonate (eg. foliar spray can reduce root rot) 3. Metalaxyl/phosphonate 4. Holistic system (managing farming systems to build biologicals, managing timing of green manures etc as initially pythium will increase on them before decreasing over time) 5. Temperature management 6. Better use of chemicals (ie. less chemicals and at appropriate times only)	1. Fungicide efficacy and use patterns 2. Biological controls 3. Non-chemical strategies eg. composts, SARs, biofumigation 4. Extension 5. Nursery hygiene 6. Seed treatments 7. Disease thresholds vs environment** 8. Hydroponics (eg. cooling of water)

*** The likely effectiveness of this strategy was queried in subsequent group discussion. Given the potential for pathogen numbers to increase from almost nothing to huge numbers in a very short time some participants felt that this would not be an effective strategy*

1.20 Appendix 1.2 – 'Best practice' IPM strategies for key soilborne pathogens in Australia. Research pirorities fitted against project modules

1.20.1 Sclerotinia

Eradication of pathogen survival structures	Disruption of the infection process	Strengthening of the host crop against disease	Exploiting host resistance to control disease
*Fungicide use (reduce spray number and improve application methods for existing chemicals, evaluate products in IR4 program, consider fluazinam +/- wetter) Develop fungicide resistance management guidelines (will require increase in the number of registered products) *Fumigant DMDS for severe infestations *Direct treatment of sclerotia on crop residues (eg. application of biologicals *S. sclerotivorum* or *C. minitans* , limes or fungicides immediately after harvest) Volatiles including (biofumigants) from organic matter , crucifer derived ITCs, DMDS, and DADS	#Disrupting and silencing key pathogenicity factors (eg Gene silencing of melanin pathway enzyme and disruption of the pathway for melanin production) Soil surface modification (as barriers to infection of leaves and inhibition of apothecial production eg. lime, Perlka, films, biological, physical, mulches etc) Transmissible hypovirulent strains of *S. minor* Inhibition of apothecia of *S. sclerotiorum* with chemicals/herbicides	Salicylic acid systemic acquired resistance (including induction by analogues INA and BTH) *Silicon mediated systemic acquired resistance Minimising crop conditions that favour disease (*S. sclerotiorum*) eg. row orientation, irrigation manipulation, fertiliser, shortening flowering period Use of biological controls eg. Contans™ as a foliar spray on bean flowers Manipulation of plant surfaces to produce sub-optimal conditions for infection. (eg. relationship between petal/plant pH and susceptibility of bean varieties to infection, increasing pH in fungicide mixes to above pH 7 for foliar applications	Screen plants for resistance, including overseas lines Selection of bean varieties for short flowering periods and less dense canopies Consider the use of GM mechanisms and crops Antifungal proteinases Oxalate oxidases

Crop rotation with broccoli as preceeding crop Germination stimulants (identify) Rouging (for lettuce varieties where bulk of sclerotes form on leaves, not roots Plant extracts (eg. oils – oregano, fennel, rosemary, thyme, garlic etc)		Coordination of timing of application with flowering (eg. at which point during flowering, when is it too late)	

* Work under way subprogram 2.1, # Work under way subprogram 2.2, ~Work under way – other research programs

1.20.2 Pythium

Eradication of pathogen survival structures	**Disruption of the infection process**	**Strengthening of the host crop against disease**	**Exploiting host resistance to control disease**
~Fungicides (efficacy, particularly in greenhouses, reduced spray number, timing, metalaxyl/phosphonate, seed treatments for damping off diseases) Improve nursery hygiene #Chitin - antifungal chitinases Mycoparasitism by non transmissible hypovirulent strains (non pathogenic *Pythium*) eg. *P. oligandrum, P. achanticum, P. nunn, P. mycoparasiticum, P. periplocum, P. monospermum* and *P. acanthophoron* Biofumigation and green manures ~Biocontrols *Bacillus subtilis* and *Streptomyces* ~~Holistic management systems to build biologicals and promote disease suppression	#Application of surfactants and biosurfactants Manipulation of pH (*P. sulcatum* increase to 7.2) ~Biocontrols *Bacillus subtilis* and *Streptomyces* Cultural controls Irrigation management (avoidance of wet conditions at planting) Temperature management (reduce soil temperature for capsicum by changing from black to white plastic mulch, cooling water in hydroponic systems)	# Chitin mediated systemic acquired resistance Silicon mediated systemic acquired resistance (cucurbits, early application) Phosphonate mediated systemic acquired resistance (may also have direct fungitoxic effect) Induced systemic resistance – *Pseudomonas* spp. Increased calcium (cucumber and carrot)	

Work under way subprogram 2.2, ~Work under way – other research programs (Tesoriero and Forsyth, NSW DPI), ~~Work under way – other research programs (Stirling et al, Biological Crop Protection QLD)

1.20.3 Fusarium

Eradication of pathogen survival structures	**Disruption of the infection process**	**Strengthening of the host crop against disease**	**Exploiting host resistance to control disease**
~Chemical seed dressings Farm hygiene ~Greenhouse hygiene Plant volatiles (biofumigants) from organic matter (particularly crucifer derived ITCs) Plant extracts (eg. pepper/mustard, clove or cassia) Water source treatment (hydroponics)	Microbial competition by non-transmissible hypovirulent strains of *F. oxysporum* Manipulation of pH (increase to 6.5-7), *F. oxysporum* in cucurbits ~Biological controls eg. *Streptomyces* ~Delay sowing to avoid periods optimum for infection (near wilt snow pea)	Host crop nutrition (supply N as NO_3^- rather than NH_4, increase K (wilts), increase B) Salicylic acid mediated systemic acquired resistance (including analogues BTH and BABA). Chitin mediated systemic acquired resistance. Silicon mediated systemic acquired resistance. Induced systemic resistance – *P. fluorescence, Serratia marcescens* and non-transmisible hypovirulent strains of *F. oxysporum* against Fusarium wilt.	#Grafting (cucurbits and snake bean, anything with low density plantings where conidia of Fusarium not significantly airborne) Tolerant (cucumber) or resistant varieties (pea)

Work under way subprogram 2.2, ~Work under way – other research programs (Tesoriero and Forsyth, NSW DPI)

1.20.4 Rhizoctonia

Eradication of pathogen survival structures	**Disruption of the infection process**	**Strengthening of the host crop against disease**	**Exploiting host resistance to control disease**
~Evaluation of fungicides for protection of brassica seedlings at the early stage of infection ~~Chemical control – targeting different AG groups with a range of active ingredients Mycoparasitism by non transmissible hypovirulent *Pythium* strains (non pathogenic *Pythium*) eg. *P. oligandrum* *Crop rotation (influence on soil inoculum loads and disease, length and cropping sequence) *Development of diagnostics to assist paddock selection	Microbial competition by non-transmisible hypovirulent strains of *R. solani*	Host crop nutrition (supply N as NO_3^- rather than NH_4, calcium nitrate at tuberset for potatoes, increase P, increase S, increase B) Salicylic acid mediated systemic acquired resistance Chitin mediated systemic acquired resistance *Seed health (importance of seed vs soilborne inoculum for different AG groups, impact of seed storage) Increasing vigour by UV treatment of seed pieces (potatoes)	High vigour varieties (faster shoot and root growth) Ranking varieties for resistance

Work under way subprogram 2.2, *Work under way HAL potato program, ~Work under way – other research programs (Hitch and Hall, SARDI), ~~Work underway – other research programs (Cathy Todd, University of Adelaide).

IPM strategies for field grown vegetable crops

Best Practice IPM strategies for the control of soilborne diseases in vegetable crops

2. IPM strategies for field grown vegetable crops

(The influence of rotation and biofumigation on soilborne diseases, yield and soil in vegetable production in Victoria).

Researchers: Oscar Villalta, Caroline Donald, Cassie Scoble, Denise Wite, David Riches, Scott Mattner, Rod Jones, Gavin Rose, David Allen and Ian Porter, Department of Primary Industries, Victoria.

In-vitro, pot and field trials were conducted to determine the influence of crop rotation and biofumigation on management of soil-borne pathogens, yield and soil properties in vegetable production in Victoria. This work has:

- identified four new brassica green manure crops (Caliente 199, Mustclean, Gladiator and Nemfix) with biofumigant activity against four major soil-borne pathogens of vegetables and confirmed that biocidal activity of these crops appears to be related to the amount of tissue and concentration of glucosinolates (eg 2-propenyl-GSL) within the tissue.
- identified Caliente 199 (completely biocidal at the lowest rate 0.25 g/plate), Nemfix, Gladiator and Mustclean (completely biocidal at the higher rate 0.5 g/plate) as the most effective treatments in in-vitro studies. These four brassica treatments, which produced the highest concentrations of volatile GSL were as good as or superior to the standard treatment Fumafert® and BQ Mulch™ and were more effective than another seven brassica treatments evaluated, which produced low or no 2-propenyl-GSL.
- described the different sensitivity of four key soilborne pathogens to isothiocyanates (ITC) compounds released from biofumigant tissue. This differential sensitivity indicates that it might be feasible for growers to select biofumigant crops to treat paddocks based on their efficacy against a specific pathogen.
- confirmed the biofumigant potential of Nemfix, Gladiator, Caliente 199 and Mustclean for controlling *Sclerotinia minor* infection and Nemfix and Mustclean for controlling *Rhizoctonia solani* in pot trials.
- demonstrated significant in-field effects of soil amendment with brassica green manures including excellent weed suppression, reduction of root rots in green beans following soil amendment with Mustclean and Caliente 199, and an increase in the fresh weight of spring onions following soil amendment with Caliente 199. The legume crop faba bean also significantly reduced root rots in green bean however, soil amendment with faba bean significantly increased levels of *Pythium* clade f in one field soil. This group contains both non-pathogenic and pathogenic species.
- determined which green manure crops, including biofumigant brassica crops are hosts of soilborne pathogens studied. Brassica green manure crops were susceptible to *P. brassicae* (clubroot). *Sclerotinia minor* and *Rhizoctonia solani* were also able to grow and reproduce on fresh tissue of some brassica species. This demonstrates that some green manure crops could be a substrate for these pathogens and thus a poor break crop choice for fields with high levels of these pathogens. In particular growers should avoid growing blends of *Brassica juncea* green manure crops in fields infested with clubroot.

Preliminary results from this on-going study indicate that soil biofumigation with brassica green manure crops and crop rotation could be useful tools to manage soil-borne pathogens and increase productivity in vegetable production in Victoria. For optimum benefit, rotation must be integrated with other IPM practices and tools throughout the rotation.

2.1 Introduction

Soil-borne diseases are persistent and costly problems for growers in intensive vegetable production. In most production systems in Victoria, vegetables are cropped year after year mostly without a break. The problem is that many of the crops rotated in the same field are often susceptible to the same soil-borne pathogen such as *Sclerotinia minor* and a multitude of other soil-borne pathogens that cause root infections. In the long term, success controlling soilborne diseases is more likely to be achieved if a more holistic (systems) approach to managing soilborne pathogens is adopted. This approach includes the use of beneficial farming practices including crop rotation and biofumigation that reduce inoculum carry-over in soil and thus the risk of disease outbreaks, supported by effective and environmentally friendly IPM tools.

There has been little research on rotations and biofumigation in vegetable cropping in both Victoria and Australia (Villalta *et al.* 2004). Particularly, there is lack of knowledge of how crop rotation practices influence disease management, yield and soil biology in vegetable production in Victoria. Most research on the potential of *Brassica* crops to control pathogens in Australia has been focused on field crops and potatoes. There are many reports in the literature showing that Brassica tissue can inhibit bacterial, fungal and nematode pathogens in laboratory and the glasshouse (Mattner *et al.*, 2008, Morra and Kirkegaard 2002, Smolinska and Horbowicz 1999). Kirkegaard *et al.* (1996) demonstrated that volatile compounds released from freeze dried root and shoot samples of canola (*B. napus*) and Indian mustard (*B. juncea*) inhibited the growth of several important cereal pathogens. There is also evidence of the possible role of biofumigation in controlling take-all in cereal in Australia (Kirkegaard *et al.* 1998). Hao *et al.* (2003) reported that rotations with broccoli can be a practical management strategy for *S. minor* lettuce drop in California because crop residue of broccoli reduced both sclerotia in soil and disease incidence.

The objectives of this study were to:

1) evaluate the influence of crop rotations with biofumigant and other green manure crops on soil-borne pathogens, disease, yield and soil in different vegetable cropping systems in Victoria; and
2) evaluate the biofumigant potential of existing and new brassica manure crops for controlling soil-borne pathogens and root diseases of vegetables in controlled experiments and in the field.

The research reported here is part of an on-going study and involves:

- laboratory, glasshouse and field trials to investigate the biofumigant and agronomic potential of existing and new brassica green manure crops for managing soil-borne pathogens in vegetable cropping;
- long-term field trials in which different crop rotation schemes with biofumigant and other green manure crops are being evaluated to control *Sclerotinia* and other pathogens causing root rots including *Fusarium* spp., *Rhizoctonia* spp. and *Pythium* spp.; and
- host susceptibility studies to explore how biofumigant and other green manure crops are influenced by soil-borne pathogens

2.2 Materials and Methods

2.2.1 Plant treatments

Table 2.1 describes the green manure treatments evaluated in laboratory and pot trials. Seed of brassica treatments was supplied by J. Kirkegaard (CSIRO, Canberra), with the exception of Gladiator, Caliente 199 and Mustclean which was obtained from seed suppliers in Victoria and New Zealand (Caliente 199). Table 2.2 describes the brassica and non-brassica green manure crop treatments evaluated in replicated field trials. Seed was obtained from seed suppliers in Victoria. Plant material in table 2.1 was grown in replicated large pots (50 L) in the glasshouse and in small plots in a sand bed outdoors at Knoxfield. Plant biomass (fresh and dry weight) and glucosinolate (GSL) content was determined on plant material grown in pots, sand bed and field trials. In the field trials, plant biomass and weed density were measured inside one square meter quadrat in each plot prior to incorporation into soil and

after drying in an oven at 50ºC. The growth habit, flowering times and various agronomic aspects were also recorded.

Table 2.1. Brassica species/varieties grown in pots and sand bed evaluated in laboratory and pot trials.

Treatment	Species/variety	Origin
Standard control	Fumafert™ seed meal	Organic Crop Protectants Pty Ltd
Addagio - Fodder radish	*Raphanus sativus*	Nematode resistant, Germany/PHPetersen/Schlatholter
Nemat	*Eruca sativus*	Biofumigant - Italy/Triumph Italia Spa/ Patalano-Lazzeri
Maxima-Plus	*Brassica napus*	Fodder rape - Kirkegaard
BQ-Mulch™	*B. napus/campestris*	Rape turnip, Biofumigant, NZ/Wrightson
Idagold White mustard	*Brassica juncea*	Oilseed (meal for biofumigant) USA/Uni Idaho/Morra
Arid	*Brassica juncea*	Low-GSL oilseed mustard Canada/Kirkegaard
Nemfix	*Brassica juncea*	Biofumigant, Australia, Seedmark Robertson
Mustclean	*Brassica juncea*	Graham's Seeds
Caliente 199	*Brassica juncea*	Biofumigant, USA, D. Gies, Andrew Culley F & S services
Gladiator	*Brassica juncea*	Biofumigant, Australia, Jacobs

Seed supplied by J. Kirkegaard (CSIRO, Canberra), except Caliente 199, Mustclean and Gladiator. Adios (fodder radish), Architekt (*B. alba or B. hirta*, white mustard) and Doublet (fodder radish) supplied by SeedForce are blends currently being tested *in vitro* experiments.

Table 2.2. Brassica and non-Brassica green manure treatments evaluated in field trials.

Treatment	Rate	Source
Fallow		
Mustclean	8-10 kg/ha	Graham's Seeds, Vic
B.Q. Mulch™	8-10 kg/ha	Wrightson Seeds, Vic
Caliente 199	8-10 kg/ha	High Performance Seeds, USA
Faba beans	80-90 kg/ha	Graham's Seeds, Vic
Triticale (Monstress)	80 kg/ha	Graham's Seeds, Vic
Ryegrass (Tetila-USA)	25 kg/ha	Graham's Seeds, Vic
Rye-corn	75 kg/ha	Graham's Seeds, Vic
Vetch (Popany)	80 kg/ha	Graham's Seeds, Vic
Oats	80 kg/ha	Graham's Seeds, Vic
Sudan grass		(used at Heatherton only)

2.2.2 Glucosinolate (GSL) concentrations

The concentrations of GSL in brassica plant material used in laboratory experiments, pot trials and crops grown in field trials was estimated using HPLC. Plant samples were collected from pots and sand bed when plants were at full flowering. Samples from field plots also were collected (5-6 reps/treatment/site) when plants were at full flowering at three field trials (Lindenow, Clyde north and Clyde south). Root and shoot material were separated from a sample of whole plants and placed into cotton bags and stored in a freezer at -20ºC then freeze dried. Freeze-dried material was pulverised using an electric grinder and then stored in sealed plastic jars until used. GSL concentrations were determined using the method of West *et al.* (2002). Only results for 2-propenyl-GSL (Sinigrin) are reported. Other GSL compounds are currently being determined and will be reported later.

2.2.3 Pathogen suppression *in vitro*

A series of laboratory experiments was conducted to test the effects of volatile hydrolysis products released from defatted mustard seed meal tissue (Fumafert®) and from freeze dried root and shoot tissue, collected from glasshouse and field (Clyde south) grown brassica plants (Table 2.1 and 2.2), on the growth of four fungal pathogens isolated from vegetable farms. The methods were based on those of Mattner *et al.* (2008) and Kirkegaard *et al.* (1998). In total ten brassica treatments were evaluated in petri dish assays for their ability to destroy or inhibit mycelial growth of *Sclerotinia minor*, *Fusarium oxysporum*, *Pythium dissotocum* complex and *Rhizoctonia solani* AG-1. Fumafert® (66 % mustard seed meal of *B. juncea* and 33.3 % Neem kernel *Azadirachia indica*) was used as a standard control.

Freeze dried brassica tissue in amounts of 0.25 and 0.50 grams was placed into one side of split petri plates. Sterile distilled water was then added to the tissue in each plate at volumes required (1.0-2.0 mL/plate) to produce a paste or slurry, with 1.0 ml added to plates with no tissue. A 5 mm plug of a 4-6 day-old culture of each pathogen was then placed on the other half of the plate containing potato dextrose agar (PDA). The plates were sealed with parafilm to prevent escape of volatile products and incubated in the dark at 20ºC. The radial growth of the fungus was measured by taking two radial transects of the colonies at various times after adding water to hydrolyse the brassica tissue. PDA plugs that did not show any mycelial growth after 7 days incubation were transferred to fresh PDA to check their viability. The mycelial growth of each fungus in the presence of treatments was compared to growth of controls without brassica tissue to determine treatment effects (inhibition or biocidal activity).
The experiments were designed as replicated complete randomised blocks and analysed by ANOVA using Genstat for Windows 12th edition (Lawes Agricultural Trust, Rothamsted Experimental Station).

2.2.4 Host susceptibility studies

Three pot trials were conducted to determine the susceptibility of brassica and non-brassica green manure treatments, being evaluated in laboratory and field trials (Table 2.1 and 2.2), to isolates of two *Sclerotinia* pathogens (*S. minor*, *S. sclerotiorum*) and one of *R. solani*. As a first step, a series of petri dish experiments were conducted to test the commercial seed used in pot and field trials for the presence of pathogenic fungi using three selective media (PDA, V8 and WA).

Inoculum of the three pathogens was prepared on autoclaved wheat and barley grain inoculated with culture pathogens and incubated for 3 weeks at 20ºC before use. Isolates of *S. minor, S. sclerotiorum* and *R. solani* used were isolated from soil and infected plants from vegetable farms in Victoria. Vegetable seed raising mix (Biogro, Bayswater Victoria) was inoculated with inoculum bulked up in grain at 1 % inoculum w/w and included un-inoculated and substrate controls. The commercial mix was steam sterilised before adding the inoculum. The fungus in the colonised grains consisted mainly of mycelium. The number of seed sown in each pot was determined by their relative sizes. For *Sclerotinia* spp. and *R. solani* trials, five seeds were sown in each pot. The pots were arranged on glasshouse benches using a complete randomised block design, with four replicate pots for each treatment/pathogen combination. The number of infected and healthy plants in each pot was assessed periodically for 30 days after inoculation. Plant density reduction due to infections was then estimated based on the number of healthy plants that grew in the respective untreated controls to compensate for un-germinated seeds.

2.2.5 The effect of brassica treatments on pathogen control in pots

Two pot trials were conducted to determine the effects of brassica treatments on the control of *S. minor* infection on green beans and *R. solani* (damping off) on broccoli seedlings.

Inoculum for both pathogens was prepared as previously described. Isolates of *S. minor* and *R. solani* used were from soil and infected plants from vegetable farms in Victoria. Vegetable seed raising mix (Biogro, Bayswater Victoria) was inoculated with inoculum bulked up in grain at 1 % inoculum (w/w) and included untreated and substrate controls. The fungus in the colonised grains consisted mainly of

mycelium. The commercial potting mix was steam sterilised before adding the inoculum and brassica treatments. The potting media with inoculum was then amended with macerated fresh tissue from eight brassica treatments (Table 2.1) at rates of 5 % and 10 % of soil (w/w). The plant material used was grown in small plots in the sand bed at Knoxfield and harvested at full flowering. Whole plants were placed in a blender to macerate the tissue so that when it was mixed with soil and rolled the sap would be released into soil. Potting mix was amended with macerated whole plant tissue at 5 % and 10 % w/w. Fumafert® was used as the standard control. Five seed of green bean (cv 'Valentino') and five of Broccoli (cv 'Marathon') were sown in each pot. The experimental design and assessment methods were similar to those described for the host susceptibility pot trials.

2.2.6 Field trials - influence of crop rotation and biofumigation on inoculum, disease, yield and soil condition

Four rotation trials were established in commercial farms in Victoria to determine the feasibility and benefits of incorporating green manure crops in between vegetable crops for the long-term management of soil-borne pathogens of vegetables. In particular the trials evaluated the impact of single crop species (legume, cereal, grass or brassica) on pathogen populations in soil, disease incidence, yield and soil condition over 2-3 year crop rotation cycles.

Field sites and treatments

The rotation trials began in early 2009. The first trial was established in eastern Victoria at Lindenow in a silty clay loam (alluvial) soil, which has a hard pan and a poor structure and drained slowly when wet. The other three trials were established in the coastal areas of Melbourne in sandy soils (Clyde south and Heatherton) with low fertility and in a clay loam soil which has poor structure, formed a hard surface crust when dry and drained slowly when wet (Clyde north). Preliminary results (1st year rotation) from three of the four sites that had commercial crops in experimental plots are reported here. Data from the fourth trial site (Clyde north) is not presented as the crops were planted too late in the season, emerged poorly and grew slowly over winter. This trial provided useful agronomic information about acceptable planting time but was not continued due to the low biomass of the green manure and biofumigant crops.

Treatments

At the four sites, four types of green manure crops (brassica, legume, cereal and grass – Table 2.2) are being rotated with crops susceptible to several soil-borne pathogens including lettuce (*S. minor*), green beans (*S. sclerotiorum, Fusarium spp., Pythium spp., Rhizoctonia spp.*), brassica speciality vegetables (clubroot) and spring onions and parsley (*S. cepivorum*, *Pythium* spp.) in 1-2 year crop rotation cycles.

BQ Mulch™, Caliente 199 and Mustclean were the brassica treatments investigated. These varieties were selected in plant breeding programs for high isothiocyanate (ITCs) production. The first is a fodder rape and the latter two are mustards. The non-brassica green manure treatments were two legumes (faba bean and vetch), one grass (rye-grass) and three cereal crops (rye-corn, oats and triticale). At all sites break and commercial crops were grown using grower's standard practices, with the exception that applications of chemical controls for the pathogens under investigation were supervised by researchers. Below is a brief description of activities at each site.

Lindenow

On the 9th of March 2009, seed of brassica and non-brassica treatments (Table 2.2) were drilled into replicated plots on a field previously in long-term rotation with green beans, sweet corn, capsicum and cabbage. The experiment with break crops was designed as complete randomised block of eight treatments including fallow and six replicates. Plots were 6 m wide and 38 m long, with a row spacing of 30 cm. Experimental plots were not fertilised. When the brassica crops reached 50 % flowering on the 20th of May (Mustclean) and on the 16 of July (Caliente 199 and BQ Mulch™), the crops were pulverised with a hammer pulveriser prior to incorporation into moist soil with a rotary hoe and mouldboard plough to a depth of 30-40 cm. On the 15th of September all plots were sown with green beans (cv 'Valentino'). Fungicide treatments (Filan™) were applied to half of each plot, the first

application on the 9th of December (15-20 % flowering) and the second on the 15th of December 2009 (>50 % flowering). The sprays were applied with the grower's boom sprayer using 300 L water/ha and a wetter (60 mL Agral/100 L water). Plots were irrigated with a pivot irrigation system. Green beans were harvested on the 26th of December.

Clyde south

Seed of brassica and non-brassica treatments (Table 2.2, oats used instead of rye-grass) were drilled into replicated plots on the 5th of April 2009. The field was in short-term rotation with spring onions, radish and parsley. The trial was design as complete randomised block of seven treatments including fallow and six replicates. Plots were raised beds 1 m wide and 40 m long, with a row spacing of 25 cm. Experimental plots were topped with poultry manure (10-15 tonnes/ha) immediately after sowing the break crops. When the brassica crops reached 20-30 % flowering on the 3rd of June (Mustclean) and 22th of July (Caliente 199 and BQ Mulch™), the crops were pulverised with a hammer pulveriser prior to incorporation into soil with a rotary hoe to a depth of 30 cm followed by overhead irrigation. On the 19th of August plots were sown with spring onions (cv 'Paragon'). Fungicide treatments (Filan®) were applied to a 10 m long section of each 40 m plot, the first applied immediately after sowing and the second 4 weeks later. The sprays were applied with the grower's boom sprayer using 700 L water/ha. Spring onions were harvested on the 19th of November 2009. Radish were sown a month later and harvested in late January 2010. Following radish, a second spring onion crop was grown and harvested in early April 2010.

Heatherton

Seed of the first three treatments evaluated (BQ Mulch™, rye-corn, sudan grass) were drilled into replicated plots on the 20th of February 2009. The other treatments evaluated (Caliente 199 and Mustclean) were sown on the 19th of May 2009. The field used was in short-term rotation with lettuce (Cos and fancy lettuce), spring onions and speciality Asian vegetables (Chinese broccoli, baby Pak Choy, etc). All treatments including fallow were replicated four times. Plots were also raised beds 1 m wide and 9 m long, with a row spacing of 25 cm. Plots were fertilised with Rustica™ at 125 kg/ha. When BQ Mulch™ reached full flowering on the 16th of March the first three crops (BQ Mulch™, rye-corn, Sudan grass) were incorporated with a rotary hoe followed by overhead irrigation. Two passes were needed to cut plants into small sections but full pulverisation of tissue was not achieved. Fifty percent of BQ Mulch™ plants were heavily infected with clubroot but plant growth (above ground biomass) was not affected. The other two treatments (Caliente 199 and Mustclean) were incorporated into soil as previously described on the 3rd of June 2009. On the 20th of August plots were sown with Chinese broccoli, which was harvested on the 19th of September 2009. This was followed by a crop of lettuce (multi-leaf butter type cv 'Emerson') which was harvested on 7th of December 2009.

Measurements

Isothiocyanate (ITC) analysis

Soil samples were collected from three field trials (Lindenow, Clyde south and north) to determine types and concentrations of ITC released into soil. The soil samples were collected four hours after brassica treatments were incorporated and stored frozen at -20ºC until analysed by Gas Chromatography (Varian 3400). ITCs measured are presented in Table 2.9. A separate report is available for method development and validation (Rose *et al*. 2010).

Inoculum

Soil samples were collected after incorporation of break crops but prior to sowing commercial crops in experimental plots to determine the effect of treatments on soil inoculum levels. The samples were collected with a hand trowel to a depth of 10-15 cm by combining ten sub-samples taken along each plot into one bulked sample.
Populations of sclerotia of *S. minor* and *S. sclerotiorum* were measured in 200 g of the soil of each plot using the wet sieving method and sclerotia viability tested on PDA media. Soil samples were also sent

to SARDI to determine levels of *Pythium* clade f and *R. solani* DNA in soil using a quantitative PCR based test to estimate pathogen DNA.

Biomass

In each trial, plant biomass (fresh weight) of green manure treatments was measured inside a square metre quadrat in each plot. Dry weights were also recorded after drying a sub-sample of plant material in an oven at 50ºC. Weed composition and weight were also measured inside each square meter.

Disease and yield

Disease incidence and severity of root rot infections were recorded at commercial harvest at Lindenow (green beans), Heatherton (lettuce, Chinese broccoli) and Clyde south (spring onions). In Lindenow, green bean plants were harvested from one square metre (3 linear metres of rows) in each plot. All plants and pods were counted to determine infections caused by *Sclerotinia* white mould. Disease incidence was recorded as the percentage of plant or pods with disease symptoms. Roots were scored for the degree of root rot infection using a rating scale of 0-5 where plants with no root infections and severe infections were assigned ratings of 0 and 5, respectively. At Clyde south, all spring onion plants were harvested from one square metre (3 linear metres of rows) in each plot and examined individually for root infection and then fresh weights and bunch numbers determined. At Heatherton, five Chinese broccoli plants were harvested from each plot and examined for clubroot infections and then fresh weights taken. For lettuce crops, all plants in plots were monitored regularly to determine the number of plants killed by *S. minor* over the crop's life. Other problems that affected marketability were also recorded at all sites.

Soil parameters

A sub-sample of the bulk soil sample collected from each plot was used to determine the effect of green manure treatments on soil chemical and biological properties. Soil microbial communities (total bacteria, fungi and actinomycetes) were determined using standard serial soil dilution plating and selective media. Soil chemical properties and nutrient levels were determined by standard procedures used by SWEP analytical laboratory. Soil compaction was assessed in all trials by measuring soil penetration resistance with a mechanical cone soil penetrometer (CP40II). Three readings were taken from each plot on soil at field capacity and then the average of soil penetration (kPa) on the top soil (0-250 mm) and sub-soil (250-450 mm) estimated for each plot. Only results for Lindenow are presented in this report.

Statistical analysis

The results were analysed by general analysis of variance (ANOVA) using Genstat for Windows 12th edition (Lawes Agricultural Trust, Rothamsted Experimental Station). Data was transformed, when required, before analysis to normalise and stabilise the variance. When significant treatment and interaction effects were determined the means of treatments and interactions were compared using LSD tests (5 %).

2.3 Results and discussion

2.3.1 Concentrations of glucosinolate (GSL) in brassica tissue

Field samples

Only results for 2-propenyl-GSL are currently available and presented (Table 2.3). At Clyde south, Caliente 199 (*B. juncea*) had the highest concentration of GSL in leaf tissue, which was significantly higher than concentrations measured in leaf tissue of Mustclean (*B. juncea*) but not in BQ Mulch™ (*B. napus/B. camprestris*). GSL levels were similar in stem tissue for the three brassica crops at this site. At Clyde north, Caliente 199 also had the highest concentration of GSL in leaf and stem tissue, which

were significantly higher than GSL levels measured in tissue from the other two brassica crops. BQ Mulch had significantly more GSL in the stem than Mustclean but not in leaf tissue. At the two Clyde sites, there were significant differences in GSL concentrations between the two tissue types. At Lindenow, there were only significant differences in stem GSL levels between the three brassica treatments. Caliente 199 had, generally, lower levels of leaf and stem GSL than those measured in similar shoot tissue at the other two sites. Plants of Caliente 199 had uneven growth at Lindenow due to poor soil structure and a severe attack by cabbage aphids. As a result Caliente 199 did not produce the same amount of biomass observed at the other two sites. This was probably the reason for not producing higher levels of 2-propenyl GSL in shoot tissue at this site. Nevertheless, on average Caliente 199 had the highest levels of 2-propenyl GSL on both leaf and stem tissue across the three sites (Table 2.3).

Pot and sand bed samples

Preliminary results from analysis of first samples of tissue from pot plants showed that shoot tissue of Nemfix (*B. juncea*) and Caliente 199 had the highest levels (72-96 µmol/g dry weight) of Sinigrin or 2-propenyl-GSL (Figure 2.1). Mustclean, BQ Mulch™, and Gladiator had lower levels of GSL, decreasing from 32 µmol/g to 8 µmol/g of dry weight, respectively. The rest of brassica material tested had very low or no GSL detected. A sample of the standard control (Fumafert®) had 149 µmol/g of dry weight.

Preliminary results from analysis of first samples of tissue from plants grown in a sand bed showed that Nemfix and Caliente 199 also had the highest levels of 2-propenyl-GSL in shoot (25-35 µmol/g) and root (6-8 µmol/g) tissue (Figure 2.2). Shoot tissue of Gladiator, Mustclean and BQ Mulch® had levels ranging from 19 to 13 µmol/g), respectively while the other brassicas did not have detectable 2-propenyl-GSL. Concentrations of 2-propenyl-GSL were relatively lower (8.3-0.7 µmol/g) in the root tissue of five brassica treatments, with Nemat, Caliente 199 and Nemfix having the greatest levels.

HPLC tests have also revealed the presence of non-sinigrin GLS in root and shoot tissue which is currently being investigated.

Table 2.3. Mean concentration of glucosinolates (sinigrin or 2-propenyl GSL) measured in leaves and stems of three biofumigant *brassica* cultivars/varieties grown in replicated plots at three field sites in Victoria. Other GSL currently being determined in shoot and root tissue.

		2-propenyl GSL (µmole/g DW)[1] Tissue		
Trial site	Cultivar (species/variety)	leaf	stem	Root[2]
Clyde south				
	Mustclean (*B. juncea*)	20.0 a	8.7 a	-
	BQ-Mulch™ (*B. napus/campestris*)	33.2 a	6.7 a	-
	Caliente 199 (*B. juncea*)	57.2 b	16.8 a	-
Clyde north				
	Mustclean (*B. juncea*)	25.5 a	5.1 a	-
	BQ-Mulch™ (*B. napus/campestris*)	30.8 a	13.9 b	-
	Caliente 199 (*B. juncea*)	74.1 b	40.8 c	-
Lindenow				
	Mustclean (*B. juncea*)	30.6 a	8.7 a	-
	BQ-Mulch™ (*B. napus/campestris*)	25.8 a	17.9 a	-
	Caliente 199 (*B. juncea*)	28.2 a	11.3 a	-
Mean of three trials				
	Mustclean (*B. juncea*)	25.4	7.5	-
	BQ-Mulch™ (*B. napus/campestris*)	29.9	12.8	-
	Caliente 199 (*B. juncea*)	53.2	23.0	-

Mean values within a column and site with the same letter are not significantly different according to LSD test (5 %).
[1] Six replicate samples used for GSL analysis, except for Lindenow where five replicates were used for BQ-Mulch™ and Mustclean.
[2] Root tissue is currently being been analysed for GSL concentrations.

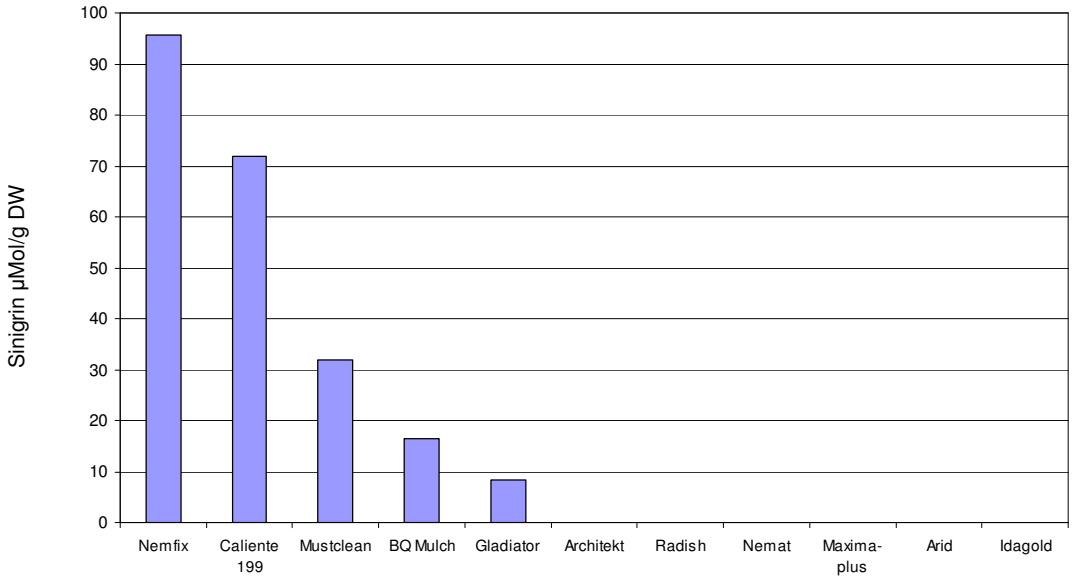


Figure 2.1. Concentrations of Sinigrin (2-propenyl glucosinolate) measured in freeze dried shoot tissue (leaves plus stems) from eleven brassica varieties/species grown in large pots in the glasshouse. (Only one sample has been analysed; Fumafert® had 149 µmol/g DW).

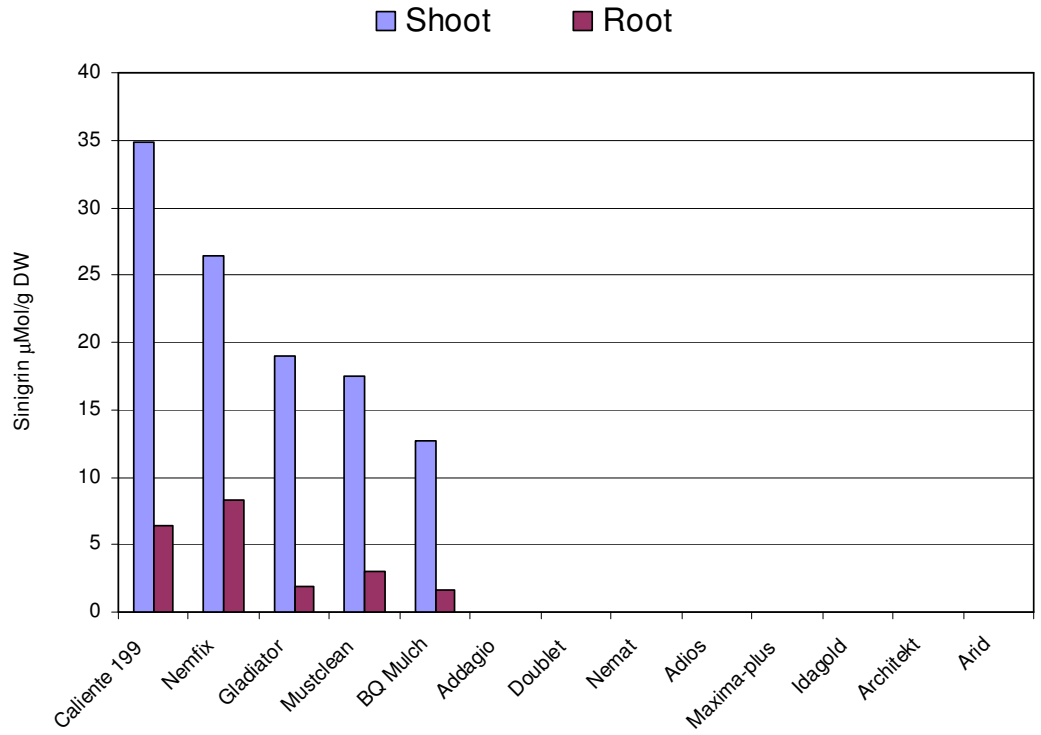


Figure 2.2. Concentrations of Sinigrin (2-propenyl glucosinolate) measured in freeze dried shoot and root tissue from twelve brassica varieties/species grown in a sand bed at Knoxfield. (Only the first sample has been analysed). Adios (fodder radish), Architekt (white mustard) and Doublet (fodder radish) has not been tested *in vitro*, pot or field trials yet.

2.3.2 The effect of brassica treatments on pathogen mycelial growth in culture

Tables 2.4, 2.5 and 2.6 show the results of laboratory experiments that investigated the effects of root and shoot tissue from ten brassica species/cultivars on mycelial growth of four pathogens (*S. minor*, *Fusarium oxysporum*, *Pythium dissotocum* complex and *Rhizoctonia solani* AG 2-1*)*. Fumafert® and oats were the GSL and non-brassica controls, respectively. Freeze dried tissue from brassica plants grown in pots was used in the experiment and included freeze dried tissue from three brassica crops (Caliente 199, BQ Mulch™ and Mustclean) grown in replicated plots at Clyde south.

Pathogen suppression

There were significant differences in the efficacy of both root and shoot tissue treatments on mycelial growth and differences in sensitivity of pathogens to treatments.

In the first test with *S. minor*, Fumafert® at 0.5g/plate was biocidal to mycelium of *S. minor* but at 0.25g/plate was only partially biocidal (not all cultures killed) and inhibitory. When testing root tissue, Nemfix at 0.5g was biocidal, but at 0.25g only partially biocidal and inhibitory to mycelial growth of *S. minor*. Other treatments (root tissue) were ineffective in inhibiting *S. minor* growth in culture (Table 2.4). There was a significant interaction between amount of root tissue and inhibitory effect, but no obvious difference in efficacy between pot and field grown root plant material for Caliente 199, BQ Mulch and Mustclean. When testing shoot tissue, Mustclean (pot only) and Gladiator at 0.5g were biocidal to *S. minor* and Caliente 199 (pot) biocidal at 0.25g and 0.5g. Caliente 199 (field), Nemfix and BQ Mulch™ (field) at 0.5g were partially biocidal or inhibitory and Caliente 199 (field), Mustclean (pot) and BQ Mulch™ (field) at 0.25g only inhibitory (Table 2.5).

In the second test with all four pathogens and using plants from Clyde south, Fumafert® treatments were biocidal to all pathogens at 0.25g and 0.5g/plate (Table 2.6). Caliente 199 was biocidal to all four pathogens at 0.5g and at 0.25g biocidal to *R. solani* but only inhibitory to the other three pathogens. Both rates of BQ Mulch™ only inhibited growth, except *R. solani.* Mustclean inhibited growth of *P. dissotocum* at 0.5g but had no effect on other pathogens. *P. dissotocum* was the most sensitive pathogen to volatile products released from shoot and also root tissue. For instance, increasing the amount of tissue from 0.5g to 1.0g/plate improved the efficacy (biocidal effect) of BQ Mulch™ on *P. dissotocum* complex but not on *S. minor* (data not shown).

Efficacy of treatments in culture

Results from this experiment indicate that isothiocyanate liberating GSL with biofumigation potential were mostly present in shoot tissue, except for Nemfix. Caliente showed the greatest potential to kill mycelium of *S. minor* at the lowest rate of tissue tested. However, shoot tissue of Caliente 199 plants grown in pots were more effective in killing mycelium than tissue from field plants. It is possible that pot grown plants, which were fertilised regularly and produced very lush and tender tissue, accumulated more GSL than field plants which had very fibrous stems and lignified plant material. Brassica treatments with the highest levels of GSL in shoot tissue (Figure 2.1) were the only treatments to show potential for biofumigation in culture (Table 2.5 & 2.6). Further testing is required to find the lowest amount of tissue required for these crops to kill mycelium and other inoculum structures such as melanised sclerotia of *S. minor*.

Plant pathogenic fungi tested appeared to have different sensitivity to the different types of ITCs released from freeze dried brassica tissue (Sarwar *et al.* 1998). Different parts of the brassica plant produce different glucosinolates, which produce different ITCs that have different properties (Potter *et al.* 1998). Leaf glucosinolates produce volatile ITCs such as 2-propenyl-ITC that are lost rapidly, whereas root GSL often produce non-volatile ITCs such as 2-phenylethyl-ITC that can also persist over long periods of time in soil. Generally, aromatic ITCs such as benzyl-ITC and 2-phenyethyl-ITC are less volatile, but more toxic, than aliphatic ITCs such propenyl-ITC and butenyl-ITC (Sarwar *et al.* 1998).

Table 2.4. Inhibition of *in vitro* mycelial growth of *S. minor* by volatile products released from freeze-dried root tissue of different *Brassica* cultivars/species grown in pots and field plots.

	Mycelial growth (mm)[1]			
	After 5 days of incubation		**After 14 days incubation**	
Cultivar (species/variety)[3]	**0.25 g/plate[2]**	**0.50 g**	**0.25 g**	**0.50 g**
Untreated	28.00 a	-	28.00 a	-
Fumafert®	6.00 c	0.00 d	7.00 b	0.00 c
Nemfix (*B. juncea*)	19.12 b	0.00 d	28.00 a	0.00 c
Caliente 199 (*B. juncea*)	27.00 a	25.62 a	28.00 a	28.00 a
Caliente 199 (*B. juncea*) - field	26.25 a	25.75 a	28.00 a	28.00 a
Mustclean (*B. juncea*)	26.50 a	27.12 a	28.00 a	28.00 a
Mustclean (*B. juncea*) - field	26.50 a	26.62 a	28.00 a	28.00 a
BQ-Mulch™ (*B. napus/campestris*)	26.88 a	26.49 a	28.00 a	28.00 a
BQ-Mulch™ – field	26.00 a	23.62 a	28.00 a	28.00 a
Gladiator (*B. juncea*)	27.38 a	26.75 a	28.00 a	28.00 a
Arid (*B. juncea*)	27.12 a	26.75 a	28.00 a	28.00 a
Idagold (*Sinapsis alba*)	26.88 a	27.12 a	28.00 a	28.00 a
Maxima-Plus (*B. napus*)	26.75 a	26.62 a	28.00 a	28.00 a
Addagio (*Raphanus sativus*)	26.38 a	26.25 a	28.00 a	28.00 a
Nemat (*Eruca sativus*)	27.62 a	26.88 a	28.00 a	28.00 a
Oats (*Avena sativa*)[3]	26.38 a	26.50 a	28.00 a	28.00 a

Mean values, within each assessment time, with the same letter are not significantly different according to LSD tests (5 %). There was a significant interaction between treatment and amount of tissuetested.

[1] PDA plug without mycelial growth after 5 days incubation were transferred to fresh PDA plates to determine if effect was fungistatic or biocidal. Red = biocidal; yellow = did not kill all cultures; green = only inhibitory.

[2] Amount of freeze dried tissue added to each petri dish (split plates).

[3] Brassica plants were grown in large pots in a glasshouse. Fumafert purchased from Organic Crop Protectants (66.6 % mustard seed meal (*B. juncea*) and 33.3 % Neem kernel (*Azadirachia indica*). Oats added as non-brassica control.

Table 2.5. Inhibition of *in vitro* mycelial growth of *S. minor* by volatile products released from freeze-dried shoot (stem+leaves) tissue of different brassica cultivars and species grown in pots and field plots.

	Mycelial growth (mm)[1]			
	After 5 days of incubation		**After 14 days incubation**	
Cultivar (species/variety)[3]	**0.25 grams[2]**	**0.50 grams**	**0.25 grams**	**0.50 grams**
Untreated	28.00 a	-	28.00 a	-
Fumafert®	6.00 d	0.00 d	7.00 b	0.00 c
Caliente 199 (*B. juncea*)	0.00 d	0.00 d	0.00 c	0.00 c
Caliente 199 (*B. juncea*) - field	7.25 c	1.38 d	28.00 a	7.00 b
Mustclean (*B. juncea*)	13.50 bc	0.00 d	28.00 a	0.00 c
Gladiator (*B. juncea*)	24.25 a	0.00 d	28.00 a	0.00 c
BQ-Mulch™ (*B. napus/campestris*)	26.38 a	6.38 c	28.00 a	28.00 a
Nemfix (*B. juncea*)	23.75 a	6.75 c	28.00 a	7.00 b
BQ-Mulch™ – field	15.12 b	4.88 cd	28.00 a	7.00 b
Mustclean (*B. juncea*) - field	25.62 a	22.50 a	28.00 a	28.00 a
Arid (*B. juncea*)	26.75 a	25.12 a	28.00 a	28.00 a
Idagold (*Sinapsis alba*)	26.38 a	27.38 a	28.00 a	28.00 a
Maxima-Plus (*B. napus*)	26.12 a	24.62 a	28.00 a	28.00 a
Addagio (*Raphanus sativus*)	27.00 a	26.00 a	28.00 a	28.00 a
Nemat (*Eruca sativus*)	26.88 a	27.12 a	28.00 a	28.00 a
Oats (*Avena sativa*)[3]	27.12 a	26.75 a	28.00 a	28.00 a

Mean values, within each assessment time, with the same letter are not significantly different according to LSD tests (5 %). There was a significant interaction between treatment and amount of residue tested.

[1] PDA plug without mycelial growth after 5 days incubation were transferred to fresh PDA plates to determine if effect was fungistatic or biocidal. Red = biocidal; yellow = did not kill all cultures; green = only inhibitory.

[2] Amount of freeze dried tissue added to each petri dish (split plates).

[3] Brassica plants were grown in large pots in a glasshouse. Fumafert® purchased from Organic Crop Protectants (66.6 % mustard seed meal (*B. juncea*) and 33.3 % Neem kernel (*Azadirachia indica*). Oats added as non-brassica control.

Table 2.6. Summary i*n-vitro* effect of volatile products released from freeze-dried shoot tissue on mycelial growth of four soil-borne pathogens compared to mycelial growth in untreated controls.

		Pathogen[2]			
Treatment[1]	g/plate	*S. minor*	*P. dissotocum*	*F. oxysporum*	*R. solani*
Fumafert®	0.25	B	B	B	B
	0.50	B	B	B	B
Caliente 199	0.25	I	I	I	B
	0.50	B	B	B	B
Mustclean	0.25	N	N	N	N
	0.50	N	I	N	N
BQ Mulch™	0.25	I	I	I	N
	0.50	I	I	I	I

[1] Three brassica crops were grown in replicated plots at Clyde south.

[2] Isolated from soil and infected plants collected from vegetable farms, *Pythium dissotocum* complex; *Rhizoctonia solani* AG 2.1

B = biocidal; I = inhibitory; N = no effect. Root tissue was ineffective in inhibiting pathogens.

2.3.3 The effect of biofumigant treatments on disease control in pots

Two pot trials investigated the effect of amending soil with fresh whole plant tissue from six brassica treatments on control of *S. minor* and *R. solani* infection on young green bean and broccoli plants, respectively.

S. minor

Soil amended with Nemfix, Gladiator and Mustclean at 5 % and 10 % (w/w) had the greatest survival of green bean plants, which was statistically comparable to untreated controls 30 days after germination (Table 2.7). Plant survival was significantly reduced (30-60 %) by *S. minor* in other treatments including Fumafert® and Caliente 199. The exception was the fodder radish Addagio which added to soil at 10 % (w/w) had a plant survival level similar to that in untreated controls. Pots treated with oats at 5 % had the lowest plant survival (30 %) probably due to the ability of *S. minor* to infect this tissue and thus increase inoculum. There was a significant negative interaction between two of less effective treatments and the two levels of tissue tested, except for Addagio. For instance, the percentage of plants that survived in soil treated with Fumafert® and Caliente 199 was significantly reduced by increasing the rate of tissue (Table 2.7).

Table 2.7. Survival of green bean plants in pots after treatment of *S. minor* infested soil (mycelium 1 % w/w) with fresh plant tissue from different brassica treatments.

	% survival bean plants per pot[1]	
Cultivar (species/variety)[3]	**5 % tissue w/w[2]**	**10 % tissue w/w**
Untreated control	100 a	-
Untreated control (grain only)	100 a	-
Nemfix (*B. juncea*)	95 a	80 a
Gladiator (*B. juncea*)	80 ab	85 a
Mustclean (*B. juncea*)	75 ab	80 a
BQ-Mulch™ (*B. napus/campestris*)	70 b	50 bc
Pathogen control	70 b	-
Fumafert®	70 b	40 c
Caliente 199 (*B. juncea*)	70 b	45 c
Addagio (*Raphanus sativus*)	60 bc	90 a
Oats (*Avena sativa*)	40 c	65 bc

Mean values with the same letter are not significantly different according to LSD test (5 %). There was no significant interaction between treatments and two levels of tissue tested.
[1] % of plants from total germinated that survived and grew healthy in pots during 30 days.
[2] Amount of fresh macerated whole plant tissue added to each pot with potting mix.
[3] Brassica plants used were grown outdoors in small plots with sandy soil and used when flowering. Fumafert® purchased from Organic Crop Protectants (66.6 % mustard seed meal (*B. juncea*) and 33.3 % Neem kernel (*Azadirachia indica*). Oats added as non-brassica control.

Rhizoctonia

Disease developed slowly and most broccoli seedlings in soil amended with the pathogen had some degree of *Rhizoctonia* infection including stem pruning (damping off) by day 30 after germination. Under these conditions, soil amended with Nemfix at 5 % (w/w) and Mustclean at 10 % had the greatest survival of broccoli plants which was statistically comparable to the untreated control and the pathogen control (Table 2.8). In other treatments, plant survival was significantly reduced (35-80 %) by *R. solani* compared to untreated and pathogen controls. There was no significant interaction between treatments and tissue levels tested. However, increasing the amount of tissue from 5 % to 10 % reduced the percentage of plants that survived in soil treated in some of the treatments (Caliente 199, Gladiator, Nemat and oats). It is possible that some of the brassica treatments tested are a host of this isolate of *R. solani* and this increased the amount of inoculum present which increased infection.

Table 2.8. Survival of broccoli plants in pots after treatment of *R. solani* infested soil (mycelium 1 % w/w) with fresh plant tissue from different brassica treatments.

	% survival broccoli plants per pot[1]	
Cultivar (species/variety)[3]	**5 % tissue w/w[2]**	**10 % tissue w/w**
Untreated control	100 a	-
Pathogen control	75 ab	-
Nemfix (*B. juncea*)	70 ab	60 b
Mustclean (*B. juncea*)	65 b	75 ab
Oats (*Avena sativa*)[3]	65 b	30 c
Nemat (*E. sativus*)	65 b	40 bc
Gladiator (*B. juncea*)	55 bc	25 c
Caliente 199 (*B. juncea*)	40 bc	20 c
Addagio (*Raphanus sativus*)	40 bc	35 c
Arid *(B. juncea)*	40 bc	65 b
Fumafert®	35 bc	40 bc
BQ-Mulch™ (*B. napus/campestris*)	30 c	40 bc

Mean values with the same letter are not significantly different according to LSD test (5 %). There was no significant interaction between treatments and levels of tissue tested.
[1] % of plants from total germinated that survived and grew healthy in pots for 30 days.
[2] Amount of fresh macerated whole plant tissue added to each pot with potting mix.
[3] Brassica plants used were grown outdoors in small plots with sandy soil and used when flowering. Fumafert® purchased from Organic Crop Protectants (66.6 % mustard seed meal (*B. juncea*) and 33.3 % Neem kernel (*Azadirachia indica*). Oats added as non-GSL control.

2.3.4 Field trials - the effect of rotation and biofumigation on inoculum, disease and yield

Isothiocyanate (ITC) release into soil

The concentrations of five ITC compounds were measured in soil four hours after brassica crop incorporation at Lindenow and Clyde south (Table 2.9). The compounds allyl-ITC, 2-phenylethyl-ITC and 3-butenyl-ITC were detected in soil at the highest concentrations at the two sites. At Lindenow, the crop residue of Mustclean released significantly higher levels of allyl-ITCs in soil than BQ Mulch™ and Caliente 199 which both produced statistically similar levels of allyl-ITCs. 3-butenyl-ITC was detected only in soil amended with Mustclean and BQ Mulch crop residue, and 4-pentenyl-ITC detected only in soil with BQ Mulch™. At Clyde south, Mustclean also released significantly higher levels of allyl-ITCs than BQ Mulch™ and Caliente 199. 2-phenylethyl-ITC was detected in soil amended with crop residue from all three brassica treatments but levels were only significantly higher in soil amended with Mustclean crop residue. 3-butenyl-ITC was detected only in soil with Mustclean and BQ Mulch™ residue and 4-pentenyl-ITC detected only in soil with BQ Mulch™.

Table 2.9. Concentrations of ITCs detected four hours after incorporating crop residue of three biofumigant brassica cultivars into soil at two replicated field trials in Victoria.

	ITC concentrations (mg/kg)				
Trial/Cultivar	Allyl-ITC	Benzyl-ITC	2-Phenylethyl-ITC	3-Butenyl-ITC	4-Pentenyl-ITC
Lindenow (alluvial soil)					
Caliente 199	0.194 b	0.000	0.000 b	0.000 b	0.000
Mustclean	0.578 a	0.005	0.038 a	0.092 a	0.000
BQ Mulch™	0.160 b	0.000	0.021 ab	0.187 a	0.188
Fallow	0.000 c	0.000	0.000 b	0.000 b	0.000
Clyde south (sandy soil)					
Caliente 199	0.112 b	0.057	0.235 b	0.000	0.000
Mustclean	0.183 a	0.000	0.477 a	0.031	0.000
BQ Mulch™	0.063 b	0.000	0.219 b	0.019	0.021
Fallow	0.000 c	0.000	0.000 c	0.000	0.000

Mean values within a column and site with the same letter are not significantly different according to LSD tests (5 %).

Effect on inoculum, disease, yield and soil

Four long-term trials have been established in vegetable farms in Victoria to determine the influence of rotation and biofumigation on management of soil-borne pathogens, yield and soil condition. Results from the first year of these 2-3 year rotation trials are presented.

Lindenow

This trial was located in a field with poor soil structure and cropped with green beans in a 2-yr rotation with cabbage, sweet corn and other crops. Prior to sowing the green manure treatments, soil had low levels of sclerotia of *S. sclerotiorum* (average 3 sclerotia kg soil). Culture and DNA tests also revealed that *F. oxysporum*, *Pythium* spp. including the pathogenic *P. dissotocum* and *Pythium* clade f and *R. solani* were also present in this soil (Table 2.10).

There were no significant differences in fresh weight between green manure crops prior to soil incorporation, probably due to uneven growth of crops at this site with poor soil structure (Table 2.10). BQ Mulch™ produced the highest amount of biomass per square metre (7.10 kg/m^2; 71 tonnes/ha) and vetch the lowest (3.26/m^2; 32 tonnes/ha). The three brassica crops, rye-grass and faba beans were very effective in suppressing weeds during the break period compared to other treatments (Figure 2.3). A green bean crop was grown in the experimental plots after the break crop treatments were incorporated into soil. The incidence of bean white mould was low and uneven across all plots, ranging from 0 to 2.4 % in plots that had only green manure treatments and the fallow, and from 0 to 0.9 % in plots where an integrated approach (manure plus Filan®) was tested (data not shown). The incidence of white mould was also low on bean pods (marketable yield), ranging from 0.5 % to 2.5 % on green manure treatments and from 0.09 % to 1.2 % on manure plus Filan™ treatments (data not shown). Bean plants in plots treated with Filan™ had slightly lower disease than plants in the respective untreated plots. These results indicated that the majority of infections were caused by airborne ascospores of *S. sclerotiorum*. The results also emphasised the problem in controlling this type of infection with fungicide if a low volume of water is used (300 L/ha) on a dense crop canopy even though disease pressure is low.

Most bean plants harvested had some degree of root rot infection, most likely caused by a mixture of pathogens including the two detected in soil (Table 2.10). There were no significant differences in *R. solani* levels across all plots but levels of *P. clade* f were significantly higher in soil that had crop residue of faba bean (Table 2.10). *P. clade* f contains both pathogenic and non-pathogenic *Pythium*. Green bean plants grown in soil amended with crop residue of Mustclean and faba bean had significantly reduced root rot severity compared to plants from the fallow control, rye-grass and triticale (Figure 2.4 and Table 2.10). The root rot ratings for Mustclean and faba bean were statistically similar to those of Caliente 199, BQ Mulch™ and vetch. There were no significant differences in fresh weights of plants or bean pods. However, plants grown in plots that had residue of Caliente 199, faba beans, triticale and vetch were observed to have higher plant weights than plants in the fallow and other green manure treatments (Figure 2.5).
Results from this site suggest all brassica treatments provided some degree of soil biofumigation which resulted in reduced root rot infections. From the three mustards, Mustclean gave the highest reduction probably due to the highest levels of allyl-ITCs and 2-phenylethyl-ITCs released into soil (Table 2.9). Faba beans gave the highest reduction of root infections probably due to pathogen suppression effects from enhanced nitrogen and microbial activity in soil. Amending soil with Caliente 199 also appeared to enhance plant growth. This requires further investigation.

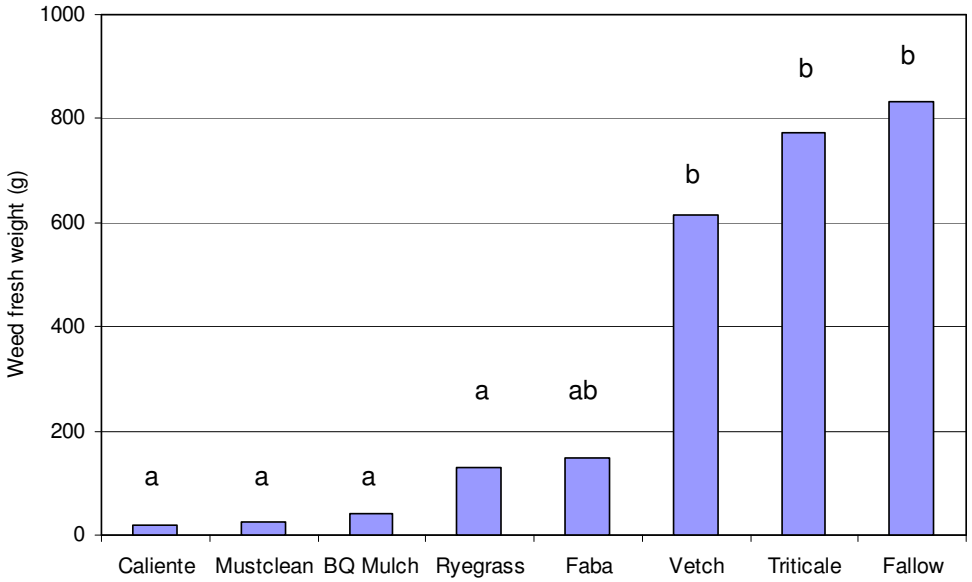


Figure 2.3. Effect of green manure crops on weed suppression per square meter at Lindenow. Means (bars) with different letters are significantly different at 5 %.

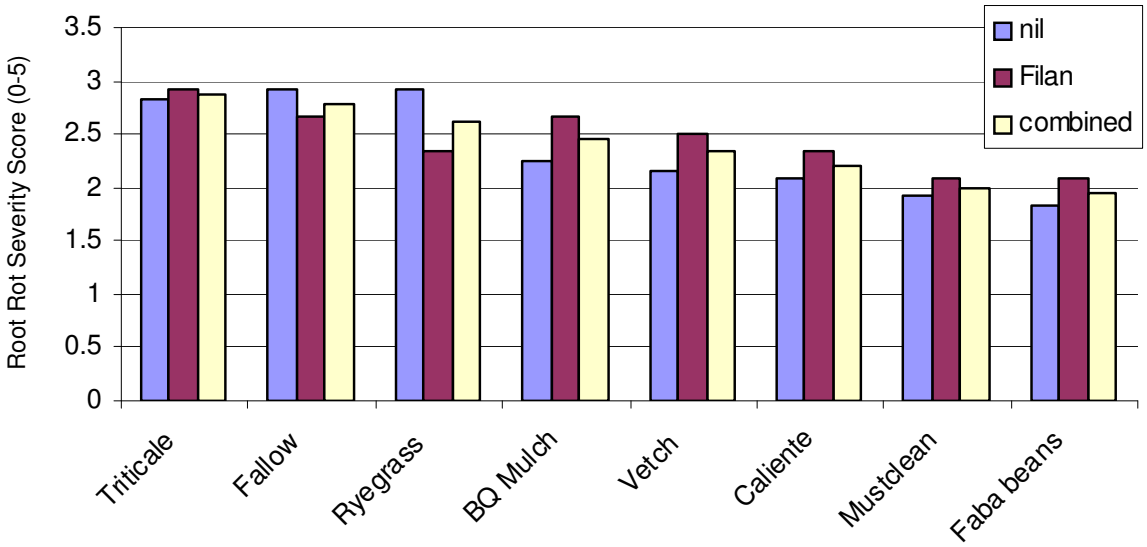


Figure 2.4. Effect of green manure treatments on root rot severity of green beans at Lindenow. Trial was designed as a split plot design with half of each plot being treated with the fungicide Filan® at flowering to protect against the aerial stage of disease. Data from fungicide treated, untreated and combined data from both types of plots is presented to show trends. Lsd values provided in Table 2.10.

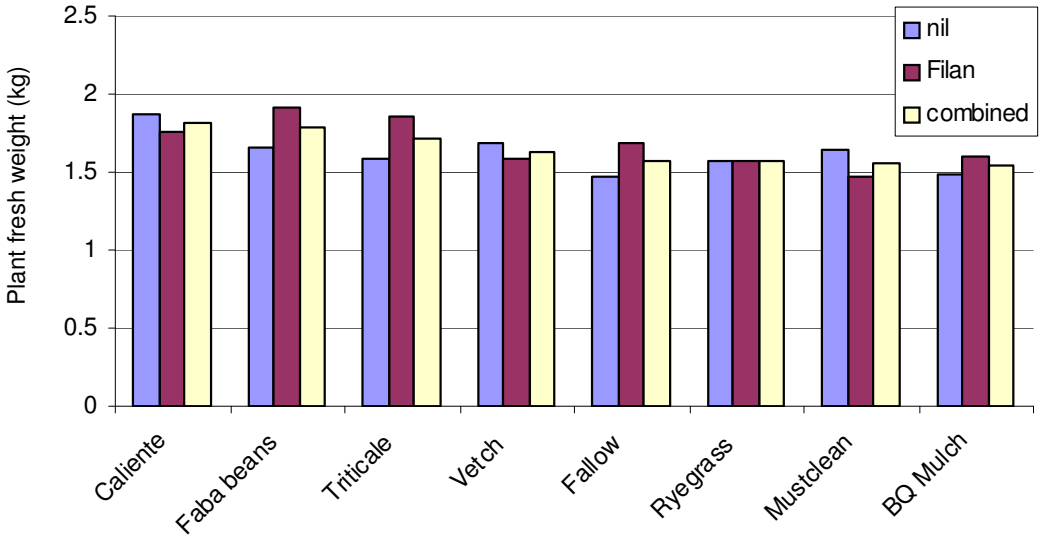


Figure 2.5. Effect of green manure treatments on mean plant weight (m^2) of green beans at Lindenow. Trial was designed as a split plot design with half of each plot being treated with the fungicide Filan® at flowering to protect against the aerial stage of disease. Data from fungicide treated, untreated and combined data from both types of plots is presented to show trends. Differences were not statistically significant.

Analysis of soil showed that there were significant differences in the levels of organic matter, nutrient levels and populations of total fungi among green manure treatments (Table 2.11, 2.12 and 2.13). Plots amended with BQ Mulch™ and Caliente 199 had the highest levels of organic matter, which was significantly higher than organic matter levels in plots with rye-grass and triticale. This field is always cropped to minimise soil erosion. This may explain the high levels of organic matter in the fallow plots, which also had the highest levels of weeds. In general, biofumigation with brassica treatments did not appear to affect negatively soil communities of actinomycetes, total bacteria and total fungi compared to levels measured in soil from fallow and other treatments (Table 2.13).

There are several interesting trends between green manure treatments and levels of macro and micro nutrients measured in soil. One is the amount of available nitrogen which was highest in fallow plots (Table 2.11). Experimental plots with green manure crops were not fertilised and as a result crops utilised ('mopped up') the nitrogen available in the soil to grow. Other relationships will be explored later using multiple linear regression analysis.
Preliminary analysis of penetrometer data also indicated that there was no difference in soil compaction in the top-soil (0-250 mm, kPa resistance penetration) among plots (Table 2.11). Brassica biofumigant crops (BQ Mulch™, Mustclean and Caliente 199) have a high biomass and very strong and deep tap root system and it was expected they would improve soil structure. Measurements of soil resistance (kPa) were high indicating that the soil at this site had poor structure. It is possible that untreated soil from the sub-soil was placed on top of soil amended with residue from green manure treatments during preparation of soil for green beans. Therefore further measurements are warranted to determine changes in soil structure once the ground is prepared again.

Clyde South trial

This trial was located in a field cropped with spring onions in short rotation with radish and parsley in raised beds with sandy soil. Two spring onion crops and a radish crop have been grown in the experimental plots after green manure crop treatments were incorporated into soil.

Green manure crops grew evenly across all plots and as expected there were significant differences in fresh weight among green manure crops. BQ Mulch™ produced significantly more biomass per square meter (11.76 kg/m^2; 117 tonnes/ha) than all other treatments (Table 2.14). Oats, Mustclean and Caliente 199 produced statistically similar levels of biomass (89-97 tonnes/ha), while faba beans (seed

planted too shallow resulting in reduced germination) and rye-corn had the lowest plant biomass. The three brassica crops were the most effective in suppressing weeds during the break period compared to other treatments (Figure 2.6). The soil had very low levels of sclerotia of *S. cepivorum* (< 2 sclerotia kg of soil). Consequently very little root rot (<1 %) including allium white rot developed on the two spring crops grown in experimental plots during spring 2009 and autumn 2010. An integrated approach combining Filan® with green manure treatments was also tested at this site for white rot control. There was no difference in disease levels among all plots indicating that the fungicide treatment could be omitted at this site because disease pressure was low.

DNA tests also revealed that presence of *Pythium* clade f and *R. solani* in soil. However there was no evidence of root infections caused by these pathogens on this particular crop grown during dry and warm periods of spring and autumn. Levels of *Pythium* clade f were very high at this site and soil amended with crop residue of Mustclean and rye-corn had significantly lower levels of this pathogen and faba beans the highest (Table 2.14). Previously the project team has observed outbreaks of root rot caused by *Pythium* sp and *Fusarium* sp. on spring onions and parsley at this site when soil is wet for long periods. Therefore, further assessments are warranted at this site.

There were no differences in bunch numbers, but there were significant differences in fresh weights (Table 2.14, Figure 2.7). Spring onions are heavily fertilised with two applications of composted poultry manure (topping) and fertiliser to compensate for the low fertility of sandy soil. Despite this, plants grown in plots with faba beans and Caliente 199 had significantly higher fresh weights than plants grown in plots with oats and rye-corn but not on the other treatments (Table 2.14, Figure 2.7). It is possible that crop residue from oats and rye-corn were not fully broken down when spring onions were sown delaying germination (phytotoxic). Results with Faba beans, Mustclean and Caliente 199 also suggest some possible biofumigation effect occurred which enhanced plant growth. Mustclean and Caliente 199 released the highest levels of two of the ITCs compounds detected at this site (Table 2.9). This also requires further investigation.

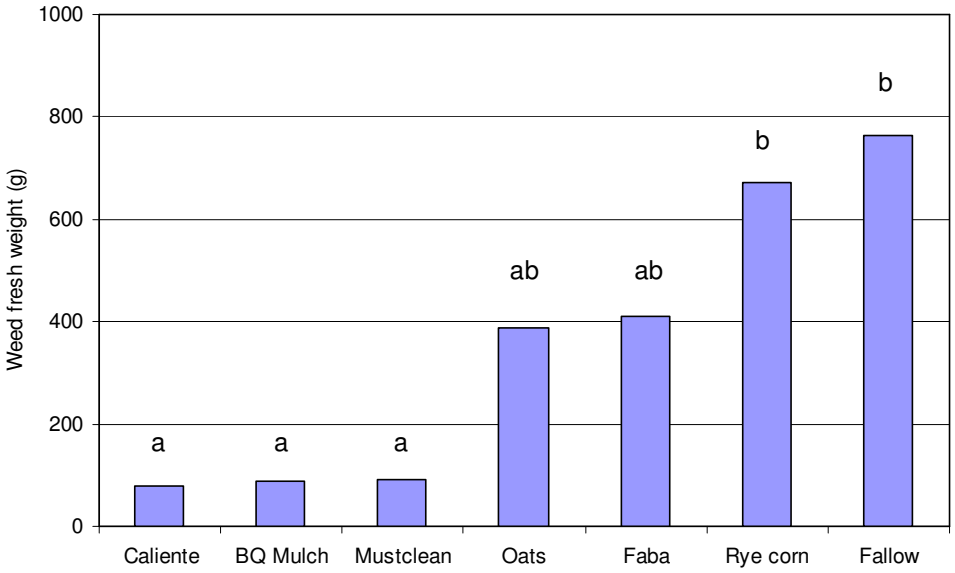


Figure 2.6. Effect of green manure crops on weed suppression (m^2) at Clyde south. Means (bars) with different letters are significantly different at 5 %.

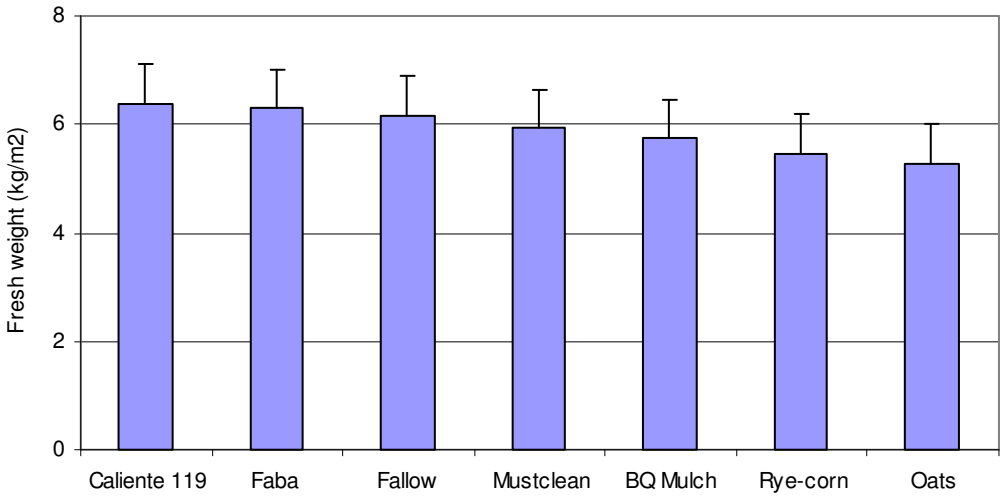


Figure 2.7. Effect of green manure crops on fresh weight of spring onions (m^2) at Clyde south. Bars above means (bars) represent lsd values at 5 %.

Analysis of soil data showed that there were some significant differences in the levels of macro and micro nutrients and populations of bacteria and fungi among green manure treatments (Tables 2.15-2.17). Plots amended with BQ Mulch™ and Caliente 199 had the highest levels of available soil nitrogen, which was significantly higher than nitrogen levels in other treatments. BQ Mulch™ and Caliente 199 produced the highest levels of biomass and this probably contributed to the high levels of available nitrogen and phosphorus in soil. Other relationships will be explored later using multiple linear regression analysis. Biofumigation with brassica treatments did not affect negatively soil microbial communities in soil (Table 2.17).

Heatherton

This trial was located in a field routinely cropped with lettuce in short rotation with speciality Asian brassica crops in raised beds with sandy soil. Three commercial crops (Chinese broccoli, lettuce and Pak Choy) have been planted in experimental plots after green manure crop treatments were incorporated into soil.

This site has a history of severe clubroot infection and soil DNA analysis confirmed that soil is heavily infested with the clubroot pathogen. BQ Mulch™ produced the highest biomass (6.65 kg/m^2; 66.5 tonnes/ha) at this site, which was significantly higher than biomass produced by rye-corn and sudan grass (Table 2.18). Caliente 199 and Mustclean were severely infected and stunted by the clubroot pathogen. Plants of Chinese broccoli grown in plots amended with crop residue from non-brassica treatments (rye-corn and sudan grass) and BQ Mulch™ had significantly lower clubroot root infection scores than plants grown in plots amended with Caliente 199 and Mustclean residue (Table 2.18). Plants in plots with rye-corn and fallow had significantly higher fresh weights than plants in other treatments, except Caliente 199.

BQ Mulch™, a blend of *B. napus* and *B. campestris,* was less susceptible to clubroot infection although plants of both species exhibited root galls typical of clubroot disease. Mustclean (*B. juncea*) and Caliente 199 (*B. juncea*) were highly susceptible to clubroot and severe root infections increased the amount of inoculum in soil. This may explain in part the higher levels of infection observed in plants grown in plots that had crop residue from Mustclean and Caliente 199. One of our research priorities is to evaluate biofumigant crops that are non-host of the clubroot pathogen (e.g. radish fodder types) for use in farms with fields infested by clubroot.

DNA soil analysis indicated that *Pythium* clade f and *R. solani* were also present in this field. Soils amended with residue of BQ Mulch™ and rye-corn had significantly more *R. solani* inoculum than soil amended with sudan grass. *R. solani* was not detected in other soils. The incidence of lettuce drop, caused by *S. minor*, was too low to allow treatment comparison on the first lettuce crop planted after

Chinese broccoli (data not shown). More assessments on lettuce crops are planned in 2010 to determine the effect of green manure treatments on *S. minor* control.

Soil analysis showed that there were significant differences in the levels of some macro and micro nutrients and populations of bacteria and fungi among the manure treatments (Tables 2.19-2.21). The data will be analysed later using multiple linear regression analysis to identify useful trends and relationships. Biofumigation with brassica treatments did not appear to negatively affect soil microbial communities (Table 2.21).

Table 2.10. Effects of green manure crop treatments on biomass, soil inoculum, root rot severity and yield of green beans at Lindenow, Victoria.

Treatment	Biomass[1] fresh kg/m^2	*Pythium* clade f (DNA pg/g soil)[2]	*R. solani* (DNA pg/g soil)[2]	Root rot[1] severity		Total plants/plot[1] kg/m^2		Yield pods[1] g/m^2	
				Combined[4] plots	Untreated[5] plots	Combined[4] plots	Untreated[5] plots	Combined[4] plots	Untreated[5] plots
BQ Mulch™	7.10	226 a	706	2.46abc	2.25	1.54	1.49	0.77	0.79
Mustclean	5.07	302 a	573	2.00c	1.92	1.56	1.64	0.69	0.72
Caliente 199	4.60	260 a	674	2.21bc	2.08	1.81	1.87	0.91	0.89
Faba beans	4.83	675 b	981	1.96c	1.83	1.78	1.65	0.89	0.84
Vetch	3.26	215 a	401	2.33abc	2.17	1.63	1.69	0.83	0.81
Fallow	-	256 a	574	2.79ab	2.92	1.58	1.47	0.81	0.65
Triticale	5.41	261 a	364	2.88a	2.83	1.72	1.58	0.83	0.76
Rye-grass	6.22	178 a	318	2.63ab	2.92	1.57	1.57	0.76	0.77
F-test	0.106	0.003	0.348	0.02	0.33	0.58	0.92	0.36	0.71

[1] Fresh weight (biomass) of green manure crops when incorporated into soil.
[2] DNA analysis conducted by SARDI (qPCR soil testing service).
[3] Root rot severity rating; 0 no disease and 5 all root severely affected by infection.
[4] Combined averages from fungicide (Filan®) treated and untreated split plots.
[5] Data averages only from plots that received no fungicide (Filan®) treatment.

Table 2.11. Effects of green manure crop treatments on soil physical and chemical properties (macro nutrients) at Lindenow, Victoria.

Treatment	Biomass[1] kg/m^2	Soil density kPa (top soil 0 – 250 mm)	pH	Org. matter (%)	CEC me/100g	Avail N ppm	Avail P ppm	Avail K ppm
BQ Mulch™	7.10	3152	6.483	4.167 a	18.02	37.0	73.5	0.577
Mustclean	5.07	3036	6.483	4.133 ab	17.71	61.3	75.8	0.588
Caliente 199	4.60	2510	6.583	4.233 a	17.79	36.0	85.0	0.637
Faba beans	4.83	2612	6.433	3.783 ab	17.31	31.4	62.7	0.532
Vetch	3.26	2470	6.367	3.800 ab	17.73	45.5	70.6	0.572
Fallow		2657	6.633	4.067 ab	17.07	66.8	71.7	0.540
Triticale	5.41	2971	6.617	3.533 b	17.94	43.3	67.6	0.533
Rye-grass	6.22	3064	6.683	3.317 b	17.47	37.1	64.4	0.473
F-test	0.106	0.184	0.821	0.041	0.865	0.102	0.416	0.875

Table 2.12. Effects of green manure crop treatments on soil micro nutrients at Lindenow, Victoria.

Treatment	Biomass[1] kg/m^2	Avail B ppm	Avail Ca ppm	Avail Co ppm	Avail Cu ppm	Avail Fe ppm	Avail Mg ppm	Avail Mn ppm	Avail Na ppm	Avail S ppm	Avail Zn ppm
BQ Mulch™	7.10	0.517	10.74	1.967	8.58	29.8	2.113	15.83	0.513	3.35	16.9
Mustclean	5.07	0.550	10.81	1.850	7.38	27.0	2.155	15.67	0.517	3.85	26.4
Caliente 199	4.60	0.533	10.25	1.883	6.23	32.2	1.910	16.00	0.593	3.63	17.4
Faba beans	4.83	0.517	10.19	1.883	6.37	33.3	2.007	16.00	0.560	3.82	12.2
Vetch	3.26	0.567	10.38	1.750	5.97	25.3	2.020	17.00	0.640	3.78	16.4
Fallow		0.583	11.06	1.767	6.55	29.2	2.032	17.83	0.645	4.00	23.5
Triticale	5.41	0.517	11.03	1.800	7.02	23.2	2.087	15.67	0.550	3.58	24.6
Rye-grass	6.22	0.500	10.69	1.800	5.83	26.5	2.010	16.17	0.517	3.45	20.5
F-test	0.106	0.438	0.932	0.292	0.151	0.914	0.459	0.831	0.162	0.877	0.851

Table 2.13. Effects of green manure crop treatments on soil microbial communities at Lindenow, Victoria.

Treatment	Biomass[1] kg/m^2	Actinomycetes CFU/g	Bacteria CFU/g	Fungi MEA CFU/g	Fungi RBA CFU/g	FDA mg fluorescein/3h/g
BQ Mulch™	7.10	8.5×10^5	5×10^7	2.7×10^5 a	1.2×10^5 a	0.245 a
Mustclean	5.07	3.7×10^5	4×10^7	1.3×10^5 bc	1.7×10^5 a	0.231 a
Caliente 199	4.60	5.8×10^5	7×10^7	2.7×10^5 a	3.1×10^5 b	0.239 a
Faba beans	4.83	7.2×10^5	3×10^7	2.2×10^5 abd	1.7×10^5 a	0.211 a
Vetch	3.26	5.4×10^5	3×10^7	1.1×10^5 cd	1.4×10^5 a	0.212 a
Fallow-A		8.9×10^5	4×10^7	1.7×10^5 abc	1.5×10^5 a	0.213 a
Fallow-B		5.1×10^5	4×10^7	1.5×10^5 abc	1.1×10^5 a	
Triticale	5.41	5.3×10^5	5×10^7	2.1×10^5 abd	1.3×10^5 a	0.160 b
Rye-grass	6.22	4.6×10^5	5×10^7	8.2×10^4 c	1.2×10^5 a	0.230 a
	0.106					
F-test		0.194	0.062	0.033	0.005	0.006

Table 2.14. Effects of green manure crop treatments on soil inoculum and yield of spring onions at Clyde south, Victoria.

Treatment	Biomass[1] kg/m^2	*Pythium* clade f (DNA pg/g soil)[2]	*R. solani* (DNA pg/g soil)[2]	Number bunches m^2	Weight bunches[1] kg/m^2
BQ Mulch™	11.76 a	1119 b	0.63	24.10	5.743 ab
Mustclean	8.65 bd	788 ab	0.00	23.29	5.921 ab
Caliente 199	9.52 b	1010 b	3.19	23.58	6.393 a
Faba beans	6.83 c	1832 c	0.00	22.87	6.293 a
Fallow		1115 b	0.00	23.47	6.157 ac
Oats	8.91 bd	989 b	0.00	22.87	5.290 b
Rye-corn	8.25 d	949 ab	1.47	23.31	5.456 bc
F-test	<0.001	<0.001	0.611	0.862	0.027

1 Fresh weight (biomass) of green manure crops when incorporated into soil.
2 DNA analysis conducted by SARDI (qPCR soil testing service).

Table 2.15. Effects of green manure crop treatments on soil physical and chemical properties (macro nutrients) at Clyde south, Victoria.

Treatment	Biomass[1] kg/m^2	pH	Org matter (%)	EC µS/cm	CEC me/100g	Avail N ppm	Avail P ppm	Avail K ppm
BQ Mulch™	11.76 a	7.317	2.017	223.7 a	8.55	8.88 a	159.7	0.775 a
Mustclean	8.65 bd	7.383	2.050	176.8 bc	9.12	5.68 bc	163.8	0.688 ab
Caliente 199	9.52 b	7.383	2.167	240.3 a	8.91	8.90 a	148.9	0.770 a
Faba beans	6.83 c	7.550	2.067	196.3 b	8.66	6.70 b	167.4	0.675 b
Fallow		7.433	1.950	179.3 bc	9.31	5.12 bc	151.2	0.682 ab
Oats	8.91 bd	7.417	1.983	172.0 c	8.20	6.23 b	159.7	0.700 ab
Rye-corn	8.25 d	7.417	2.183	169.2 c	9.70	4.93 bc	157.3	0.640 bc
F-test	<0.001	0.053	0.306	<0.001	0.066	<0.001	0.435	<0.001

Table 2.16. Effects of green manure crop treatments on soil micro nutrients at Clyde south, Victoria.

Treatment	Biomass[1] kg/m^2	Avail B ppm	Avail Ca ppm	Avail Cu ppm	Avail Fe ppm	Avail Mg ppm	Avail Mn ppm	Avail Na ppm	Avail S ppm	Avail Zn ppm
BQ Mulch™	11.76 a	0.717	4.758 a	13.17	32.83	1.788	27.00 acd	0.935 a	1.050 a	39.7
Mustclean	8.65 bd	0.733	5.278 ab	13.85	32.00	1.928	26.17 cd	0.688 bc	0.750 bc	48.2
Caliente 199	9.52 b	0.767	5.485 bc	13.02	32.33	2.010	28.00 ac	0.812 ab	1.050 a	47.1
Faba beans	6.83 c	0.783	4.928 ab	13.55	32.50	1.942	31.50 b	0.950 a	0.950 a	47.8
Fallow		0.767	5.508 bc	13.23	31.83	2.007	29.50 ab	0.697 bc	0.900 ab	46.2
Oats	8.91 bd	0.783	4.572 a	13.62	32.17	1.802	28.00 ac	0.755 bd	0.767 bc	48.1
Rye-corn	8.25 d	0.783	5.898 b	13.25	31.67	2.023	24.67 de	0.552 c	0.650 c	44.6
F-test	<0.001	0.483	0.012	0.067	0.669	0.238	<0.001	<0.001	<0.001	0.419

Table 2.17. Effects of green manure crop treatments on soil microbial communities at Clyde south, Victoria.

Treatment	Biomass[1] fresh kg/m^2	Actinomycetes CFU/g	Bacteria CFU/g	Fungi MEA CFU/g	Fungi RBA CFU/g	FDA mg fluorescein/3h/g
BQ Mulch™	11.76 a	3.9×10^6	5×10^7 a	10.3×10^4 a	14.0×10^4 a	0.1775 a
Mustclean	8.65 bd	2.2×10^6	2×10^7 bc	6.2×10^4 bc	8.0×10^4 bc	0.1605 a
Caliente 199	9.52 b	3.0×10^6	3×10^7 bd	8.2×10^4 ab	8.0×10^4 b	0.1763 a
Faba beans	6.83 c	3.4×10^6	3×10^7 bd	6.8×10^4 bc	8.1×10^4 b	0.1333 b
Fallow-A		2.7×10^6	3×10^7 bd	6.2×10^4 bc	6.7×10^4 bc	0.1742 a
Fallow-B		3.1×10^6	1×10^7 c	4.8×10^4 c	5.5×10^4 bc	-
Oats	8.91 bd	2.3×10^6	4×10^7 ad	7.0×10^4 bc	3.2×10^4 c	0.1820 a
Rye-corn	8.25 d	3.2×10^6	1×10^7 c	4.4×10^4 c	2.2×10^4 c	0.1669 a
F-test		0.316	0.011	0.007	0.001	0.006

Table 2.18. Effects of green manure crop treatments on soil inoculum, clubroot root severity and yield of Chinese broccoli at Heatherton, Victoria.

Treatment	Biomass[1] kg/m^2	*Pythium* clade f (DNA pg/g soil)[2]	*R. solani* (DNA pg/g soil)[2]	Clubroot root infection severity (1-9)[3]	Yield grams (5 plants)
BQ Mulch™	6.65 a	1550	193 a	1.000 a	359.2 a
Sudan grass	2.13 b	2251	5 b	1.000 a	365.7 ac
Rye-corn	3.73 b	2940	14 ab	1.000 a	415.7 bc
Caliente 199	-	2553	0 b	1.555 bc	391.7 abc
Mustclean	-	2101	0 b	1.700 c	369.9 ac
Fallow	-	2279	0 b	1.425 abc	417.5 b
F-test	0.003	0.532	0.003	0.011	0.047

1 Fresh weight (biomass) of green manure crops when incorporated into soil. Caliente 199 and Mustclean were heavily infected and stunted by clubroot resulting in very little biomass available for soil incorporation.

[2] DNA analysis conducted by SARDI (qPCR soil testing service).

[3] Clubroot visually assessed on a scale 1-9 where 1 refers to no clubroot, all roots healthy, 9 refers to all roots galled, no healthy roots present.

Table 2.19. Effects of green manure crop treatments on soil chemical properties at Heatherton, Victoria.

Treatment	Biomass[1] kg/m^2	pH	Org matter (%)	EC µS/cm	CEC me/100g	Avail N ppm	Avail P ppm	Avail K ppm	Avail Ca ppm
BQ Mulch™	6.65 a	6.63	3.200	381	10.77	16.8	149.7	1.177	5.91
Sudan grass	2.13 b	6.45	3.550	474	10.92	24.4	184.2	0.990	7.25
Rye-corn	3.73 b	6.50	3.350	475	8.49	27.7	158.1	0.992	5.68
Caliente 199	-	6.30	3.375	740	9.42	64.2	144.7	0.835	7.36
Mustclean	-	6.13	3.950	712	11.11	64.3	147.7	0.890	7.55
Fallow	-	6.25	3.800	776	9.41	61.4	155.1	0.920	7.65
F-test	0.003	0.061	0.014	0.109	0.078	0.006	0.037	0.001	0.002

Table 2.20. Effects of green manure crop treatments on soil micro nutrients at Heatherton, Victoria.

Treatment	Biomass[1] kg/m^2	Avail B ppm	Avail Ca ppm	Avail Cu ppm	Avail Fe ppm	Avail Mg ppm	Avail Mn ppm	Avail Na ppm	Avail S ppm	Avail Zn ppm
BQ Mulch™	6.65 a	0.925	5.91	7.750	29.2	1.650	11.00	0.873	8.35	34.45
Sudan grass	2.13 b	0.900	7.25	7.925	27.8	1.633	11.75	0.532	5.78	34.83
Rye-corn	3.73 b	0.875	5.68	7.125	28.2	1.363	9.50	0.652	4.65	32.27
Caliente 199	-	1.225	7.36	7.575	40.0	1.225	13.50	0.532	1.27	33.33
Mustclean	-	1.500	7.55	8.200	40.8	1.280	12.00	0.640	2.02	35.50
Fallow	-	1.525	7.65	7.725	50.0	1.350	11.00	0.657	2.30	34.27
F-test	0.003	<0.001	0.002	0.045	<0.001	0.048	0.027	0.154	0.012	0.246

Table 2.21. Effects of green manure crop treatments on soil microbial communities at Heatherton, Victoria.

Treatment	Biomass[1] kg/m^2	Actinomycetes	Bacteria	Fungi MEA	Fungi RBA
BQ Mulch™	6.65 a	1.3×10^6	4×10^7 ab	1.1×10^5 a	9.0×10^4 a
Sudan grass	2.13 b	2.1×10^6	5×10^7 a	1.1×10^5 a	5.2×10^4 b
Rye-corn	3.73 b	1.6×10^6	3×10^7 ab	1.2×10^5 a	7.7×10^4 ab
Caliente199	-	1.5×10^6	4×10^7 ab	0.6×10^5 b	5.3×10^4 b
Mustclean	-	1.4×10^6	10×10^7 c	1.8×10^5 c	5.6×10^4 b
Fallow-A	-	2.3×10^6	1×10^7 b	1.3×10^5 ac	12.6×10^4 ac
Fallow-B	-	1.1×10^6	5×10^7 a	1.3×10^5 ac	14.1×10^4 c
F-test		0.110	0.002	< 0.001	<0.001

2.3.5 Host susceptibility

A series of pot trials is investigating the susceptibility of the green manure crops evaluated in field trials to infection by *S. minor*, *S. sclerotiorum* and other important soil-borne pathogens in vegetable production. The aim of this work is to better understand the factors that influence and drive the infection process on green manure crops so that strategies can be designed to effectively deploy these crops in crop rotation systems.

Commercial seed

Petri dish experiments tested samples of commercial seed used in pot and field trials for the presence of pathogenic soil-borne fungi on three selective media (PDA, V8 and WA).

Samples of seed from cereal (oats and triticale) and grass (rye-corn and rye-grass) crops, generally, had significantly more seed infected, both externally and internally (seed surface sterilised), by fungi than seed from brassica and legume crops (Figure 2.8 and 2.9). Detailed microscopic examination of infected seed showed that infections were caused by a range of airborne non-pathogenic fungi and a few fungi in the genera that causes foliar diseases (*Cladosporium* sp., *Alternaria* sp. and *Stemphylium* sp.). Seed germination was very high in all samples of cereal and grass seed tested (Figure 2.10). Brassica seed did not have internal fungal infections and germination was also very high (Figure 2.8 and 2.10).

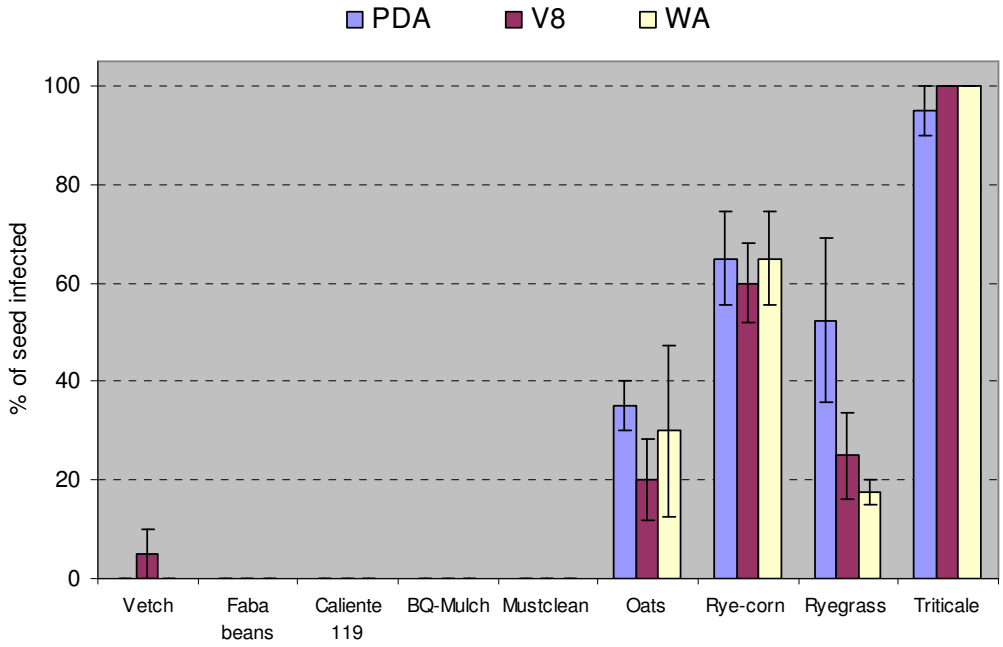


Figure 2.8. Mean percentage of surface sterilised seed from total germinated in three selective media with fungal infections (internal infections). Bars are standard error of the mean.

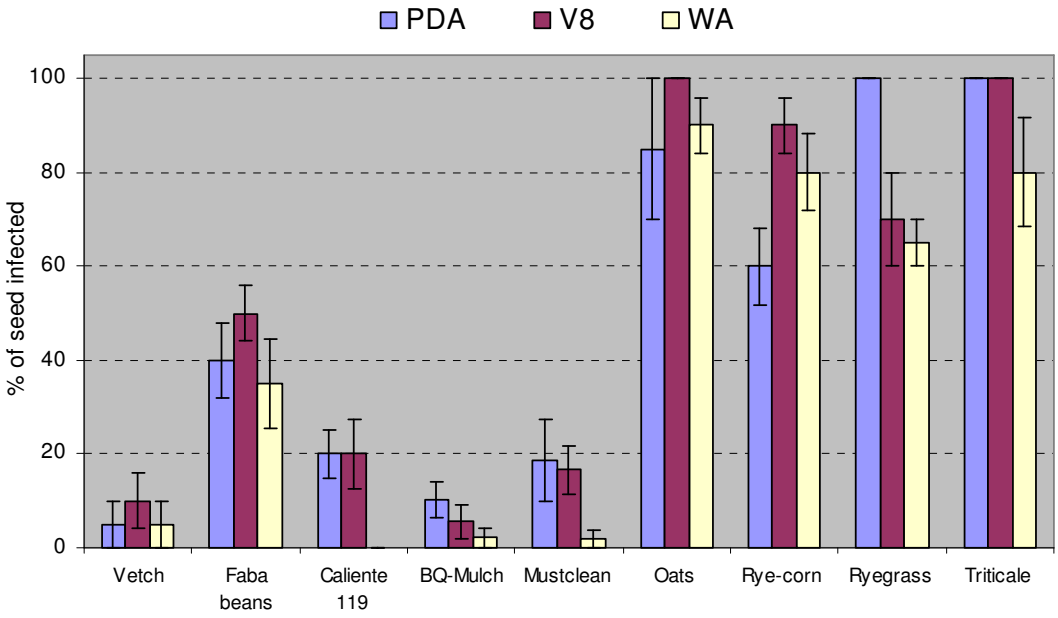


Figure 2.9. Mean percentage of seed from total germinated in three selective media with fungal infections. Bars are standard error of the mean.

.

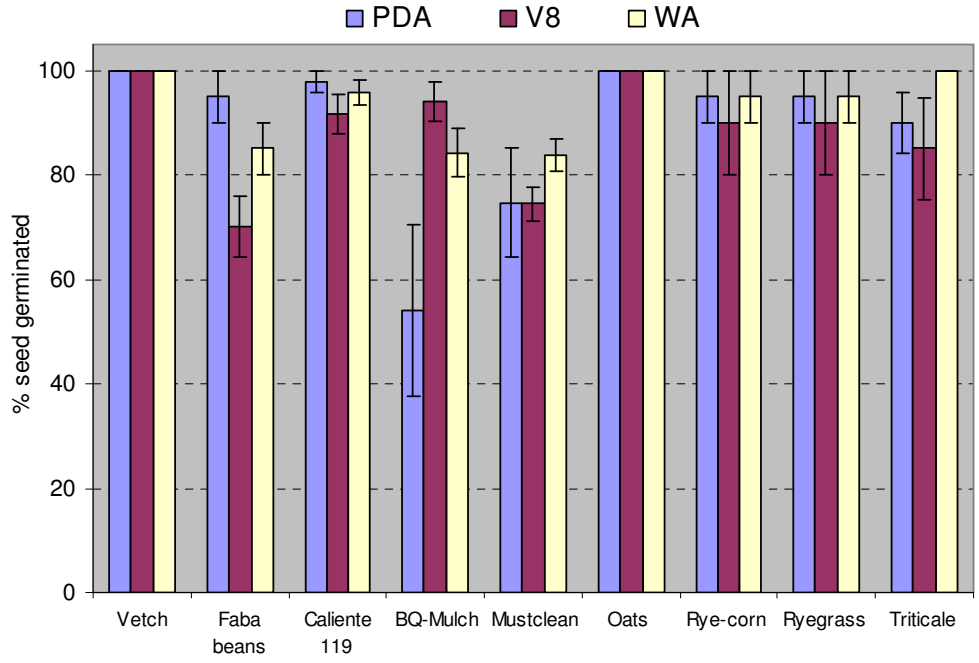


Figure 2.10. Mean percentage of total seed germinated in three selective media. Bars are standard error of the mean.

Host susceptibility

Many of the host plants tested at the seedling stage (up to 30 days) were highly susceptible to *S. minor* and *S. sclerotiorum* infection in pot soil with relatively high levels (1% w/w of soil) of mycelial inoculum (Tables 2.22 and 2.23). As expected, *S. minor* caused significant reductions in plant density (81-97 %) on *Phaseolus* and *Vicia species*. *S. sclerotiorum* also caused significant reductions on *P. vulgaris* (90 %) and *V. faba* (97.5 %) but not on vetch (*Vicia* sp.). The ten brassica crops tested were also highly susceptible to *S. minor* and *S. sclerotiorum* infection at the seedling stage under high inoculum pressure (Tables 2.22 and 2.23). All cereal and grass crops were less susceptible to *S. minor* and *S. sclerotiorum* infection. Triticale (*T. secale*) was not infected by the *Sclerotinia* pathogens and oats only infected by *S. minor*. In other cereal and grass crops the two *Sclerotinia* pathogens caused lower but significant reductions (2.5-17.5 %) in plant density in pots. Eight of the ten brassica crops tested were also highly susceptible to *R. solani* infection in pot soil with high levels of mycelial inoculum (1 % w/w) (Table 2.22).

In the pot experiments seedlings germinated in soil with abundant pathogen mycelium. The seedlings that survived appeared to be less susceptible to infection as they matured probably because shoot tissue grew away from inoculum. Levels of mycelial inoculum used in our pot trials are probably unlikely to occur in soils of most commercial vegetable farms in Victoria. For instance, populations of sclerotia of *S. minor* quantified in soils from commercial vegetable farms in Victoria were very low ranging from 1 to 7 sclerotia/kg of soil. *S. minor* and *S. sclerotiorum* survive in soil at lower densities not as mycelium but mostly as sclerotia which germinate in soil in response to exudates emitted by the roots of host plants. Inspection of green manure crops prior to soil incorporation in our field trials revealed that very few of the host (vetch and brassica crops) were infected by *S. minor* and *S. sclerotiorum.*

Table 2.22. Reduction in plant density of eight biofumigant brassica cultivars/species in soil inoculated with *S. minor* and *R. solani* (1 % w/w) 30 days after germination in pots.

	Plant density reduction (%)[1]	
Cultivar (species/variety)[3]	***Sclerotinia minor***	***Rhizoctonia solani***
Gladiator (*B. juncea*)	57.5	87.5
BQ-Mulch™ (*B. napus/campestris*)	68.3	86.7
Nemfix (*B. juncea*)	85.4	100.0
Arid (*B. juncea*)	65.0	95.0
Idagold (*Sinapsis alba*)	58.8	100.0
Maxima-Plus (*B. napus*)	65.0	100.0
Addagio (*Raphanus sativus*)	66.2	88.8
Nemat (*Eruca sativus*)	65.0	100.0

[1] Reduction calculated based on the number of plants that germinated and grew healthy in the respective untreated controls.

Table 2.23. Reduction in plant density of eight green manure break crops, including three brassica biofumigant cultivars, in soil inoculated with two soilborne pathogens 30 days after germination in pots.

Cultivar (species/variety)	*S. minor*	*S. sclerotiorum*
Green beans (*P. vulgaris*)	97.5 a	90.0 ab
Vetch (Vicia sp. Popany)	95.0 a	35.0 c
Caliente 199 (*B. juncea*)	91.7 ab	86.7 ab
BQ-Mulch™ (*B. napus/campestris*)	89.3 ab	82.0 ab
Faba beans (V. faba)	81.3 b	97.5 a
Mustclean (*B. juncea*)	76.2 b	75.1 b
Oats (*A. sativa*)	10.0 c	0.0 d
Rye-corn (*S. cereale*)	7.5 c	17.5 c
Ryegrass (*L. perenne*)	2.5 c	2.5 cd
Triticale (*T. secale*)	0.0 c	0.0 d

Mean values within columns with the same letter are not significantly different according to LSD test (5 %). Reduction calculated based on the number of plants that germinated and grew healthy in the respective untreated controls.

2.4 Conclusions

This on-going research is investigating the influence of crop rotation and biofumigation on management of soil-borne pathogens, yield and soil properties in vegetable production in Victoria. Key outcomes from the first year of laboratory, glasshouse and long-term field trials (first year rotation) are summarised below.

Four new brassica green manure crops with biofumigant activity against four major soil-borne pathogens of vegetables have been identified. Laboratory experiments showed that volatile compounds (isothiocyanates) released from freeze dried plant tissue of Caliente 199, Mustclean, Gladiator and Nemfix were inhibitory and/or biocidal to isolates of *S. minor*, *S. sclerotiorum*, *P. dissotocum* and *R. solani* collected from vegetable farms in Victoria. The biocidal activity appears to be related to amount of tissue and concentration of glucosinolates (eg 2-propenyl-GSL) with the highest levels occurring in shoots but also in root tissue for Nemfix. These four brassica treatments, which produced the highest concentrations of volatile GSL, were more effective in inhibiting or killing mycelium of pathogens than another seven brassica treatments evaluated, which produced low or no 2-propenyl-GSL. Caliente 199 was the most biocidal treatment at the lowest rate of tissue tested (0.25g/plate). Mustclean, Gladiator and Nemfix were biocidal at the higher rate (0.5g/plate) and were as good as or superior to the standard treatment Fumafert® and BQ Mulch™. Preliminary pot trials confirmed that biofumigant potential of Nemfix, Gladiator, Caliente 199 and Mustclean for controlling *S. minor* infection and Nemfix and Mustclean for controlling *R. solani*.

The four pathogens showed different sensitivity to isothiocyanates (ITC) compounds released from freeze dried tissue in petri plate bioassays, with *P. dissotocum* being most sensitive to allyl-ITCs liberated by the hydrolysis of 2-propenyl-GSL. This early result would suggest that growers could select biofumigant crops for their paddocks based on their efficacy against particular pathogen problems and agronomic characteristics. In the field, the relative susceptibility of inoculum of different pathogens to biofumigation will depend on the sturdiness of their dormant survival structures. For instance, *S. minor* occurs as melanised sclerotia, *R. solani* as sclerotia and melanised hyphae. These structures may be less vulnerable to the volatile chemicals than fungal hyphae. Therefore the next step is to evaluate the most promising brassica treatments on sclerotia.

Four rotation trials were established in commercial farms in Victoria to determine the feasibility and benefits of incorporating green manure crops in between vegetable crops for the long-term management of soil-borne pathogens of vegetables.

Preliminary results from field trials showed that amending soil with crop residues from brassica green manure crops resulted in some positive effects on disease and yield. At Lindenow, for instance, amending soil with residue of Mustclean and Caliente 199 provided significant reductions of root rot infections, caused by multiple pathogens including *P. dissotocum, R. solani, F. oxysporum* and probably others on green beans. At Clyde south, amending soil with residue of Caliente 199 significantly increased the fresh weight of spring onions. The effect of Caliente 199 on disease and yield may be related to the relatively high levels of 2-propenyl GSL produced by the foliage of this crop which were converted to high concentrations of allyl-ITCs upon hydrolysis in soil. A gas chromatography method has been successfully developed and validated to quantify the release of ITCs in soil from crop residues of biofumigant brassica crops.

Another beneficial effect provided by rotating with brassica green manure crops is excellent weed suppression.

Preliminary results from field trials also show that crop rotation is critical to the management of soil-borne diseases in vegetable production in Victoria. This was clearly shown at the Lindenow site where a minimum of 2-year rotation with green beans has prevented the build up sclerotia of *S. sclerotiorum* in soil and therefore prevented epidemics of white mould even in a crop without fungicide treatment. Short rotation with lettuce and brassica speciality crops at Heatherton has resulted in the build of clubroot inoculum in soil. This has resulted in severe clubroot infections on susceptible crops grown during warmer months of the year. The pathogens *S. minor*, *S. sclerotiorum* and *R. solani* have a wide host range and sometimes occur together in the same field. Controlling these pathogens with crop rotation is therefore difficult. This research has determined which green manure crops, including biofumigant brassica crops are hosts of soilborne pathogens studied allowing them to grow and multiply and which are poor hosts. For example, a pot study with *S. minor* and *R. solani* showed that the fungi were able to grow and reproduce on fresh tissue of some brassica species. In the Heatherton field trial, brassica biofumigants were hosts of *P. brassicae,* the cause of clubroot. This demonstrates that some green manure crops could be a substrate for these pathogens and thus a poor break crop choice for fields with high levels of these pathogens.

The impact of crop rotation on soil condition may depend on a number of factors which can have a direct or indirect effect on both pathogen inoculum and soil. The project team has also collected a large amount of soil data and determined potential beneficial effects of rotation on chemical, biological and physical properties of soil.

Preliminary results from this on-going study indicate that soil biofumigation with brassica green manure crops and crop rotation could be useful tools to manage soil-borne pathogens and increase productivity in vegetable production in Victoria. More field data is needed from long-term trials to determine the full effect of rotation with biofumigant and other green manure crops on disease, yield and soil condition. There are other factors, apart from the break crop and biofumigation, which influence disease, yield and soil. Only through on-farm trials with large plots can the impact of all variables be better understood. The next step is therefore to conduct further assessments at established field trials to continue monitoring changes in inoculum, disease, soil status and other parameters for at least two more years. More trials are also required at new sites with different soils, pathogens and cropping systems to evaluate new rotation strategies. These trials should also evaluate new biofumigant crops coming into the market, especially non-host crops to pathogens like clubroot (i.e fodder rape/radish), and investigate how rotation and biofumigation could be used as a tool to improve soil biology.

In the meantime, vegetable growers are encouraged to start trialling the new brassica green manure crops identified by this research to have biofumigant potential against soil-borne pathogens of vegetables. During cool periods of year Mustclean should begin flowering 60 days after sowing and Caliente and BQ Mulch™ 90 days after sowing. A grower in eastern Victoria has begun trialling these crops (eg Mustclean and Gladiator) and reported improvements in soil structure and water infiltration. However, this research indicates growers should avoid growing blends of *Brassica juncea* green manure crops in field infested with clubroot. Insect pest such as cabbage aphids could be a problem if brassica crops are grown during warmer periods of the year in Victoria.

This research indicates that there is scope for manipulating rotations to manage soil-borne pathogens of vegetables. But for optimum benefit, rotation must be integrated with other IPM practices and tools throughout the rotation. The challenge for researchers is to provide growers with information on suitable green manure crops including biofumigant brassica crops and rotation schedules for optimum disease management and maintenance of soil condition. The long-term goal is to identify break crops suitable for use on-farm and crop rotation sequences that minimise the build up of populations of soil-borne pathogens and weeds. Another goal is to help industry design cropping systems that maintain organic matter, soil structure and re-cycle nutrients.

2.5 Acknowledgements

We would like to acknowledge HAL and DPI Victoria for funding this work. We are also grateful to John Kirkegaard for supplying mustard seed and advice, Dale Giles for supplying Caliente 199, Jacky Edwards, Jorge Ghiano and Vijey Chandolu for helping with the experiments and James Cunnington for identification of fungi in commercial seed. We are also grateful to vegetable growers (Murray Ingram, Tony and Rocky Lammattina, Deborah and Darren Corrigan, Peter Butler and Greg Hinkins) for allowing us to conduct and help with trials in their properties. We thank Michael Imsic and Bret Henderson for helping with sample preparation and HPLC analysis.

2.6 References

Hao, J., Subbarao, K.V. and Koike, S.T. (2003). Effects of broccoli rotation on lettuce drop caused by *Sclerotinia minor* and on the population density of sclerotia in soil. *Plant Disease* **87,** 159-166.

Kirkegaard, J. A., Sarwar, M., Wong, P.T.W. and Mead, A. (1998). Biofumigation by brassicas reduces Take-all infection. *In* Proceedings of the ninth Australian Agronomy Conference, Charles Sturt University, Wagga Wagga, NSW. (Eds DL Michalik and JE Pratley) pp. 465-468, Australian Society of Agronomy.

Kirkegaard, J.A., Wong, P.T.W. and Desmarchelier, J.M. (1996) *In vitro* suppression of fungal root pathogens of cereals by *Brassica* tissues. *Plant Pathology*, **45,** 593-603.

Mattner, S.W., Porter, I.J., Gounder, R.K., Shanks, A.L., Wren, D.J. and Allen, D. (2008). Factors that impact on the ability of fumigants to suppress fungal pathogens and weeds of strawberry. *Crop Protection* **27,** 1165-1173.

Morra, M.J. and Kirkegaard, J.A. (2002). Isothiocyanate release from soil-incorporated Brassica tissues. *Soil Biology & Chemistry* **34,** 1683-1690.

Potter, M.J., Davies, K. and Rathjen, A.J. (1998) Suppressive impact of glucosinolates in *Brassica* vegetative tissues on root lesion nematode *Pratylenchus neglectus*. *Journal of Chemical Ecology* **24,** 67-80.

Rose G., Allen D. and Dyer A. (2010). Method development and analysis of ITC released in to soil treated with brassica treatments. DPI Werribee.

Sarwar, M., Kirkegaard, J.A., Wong, P.T.W., and Desmarchelier, J.M. (1998). Biofumigation potential of brassicas III. In vitro toxicity of isothiocyanates to soil-borne fungal pathogens. *Plant & Soil* **201,** 103-112.

Smolinska, U. and Horbowicz, M. (1999). Fungicidal activity of volatiles from selected cruciferous plants against resting propagules of soil-borne fungal pathogens. *Journal of Phytopathology* **147,** 119-124.

West, L., Tsui, I. and Haas, G. (2002). Single column approach for the liquid chromatographic separation of polar and non-polar glucosinolates from broccoli sprouts and seeds. *Journal of Chromatography A, **966,** 227-232.*

Villalta O., Pung H., Porter I.J. (2004). Sclerotinia final report VG00048, Horticulture Australia Ltd.

IPM strategies for soilless vegetable production systems

3. IPM strategies for soilless vegetable production systems

Researchers: Len Tesoriero and Leanne Forsyth (Elizabeth Macarthur Agricultural Institute, Industry and Investment NSW)
David Riches and Caroline Donald (Department of Primary Industries Victoria)

A range of IPM treatments were evaluated in soilless media because these production systems are also affected by several of the pathogens otherwise referred to as 'soilborne pathogens'. In particular *Pythium, Phytophthora* and *Fusarium* spp. are a significant concern in hydroponic enterprises. In addition hydroponic systems were used in this study as a rapid screening technique for novel and developing treatments. This work has demonstrated that:

- *Pythium aphanidermatum* (09/89) and *Phytophthora cryptogea* (08/174-1) were the most aggressive isolates tested on spinach while *Pythium sulcatum* was the most aggressive species to coriander.
- a single treatment of Fulzyme Plus™ (*Bacillus subtilis*) plus Phosphorous acid (Phoscare®) significantly suppressed disease symptoms caused by *P. sulcatum* in coriander. *Bacillus subtilis* also stimulated plant growth.
- a liquid chitin formulation and chitosan treatments consistently reduced the severity of disease caused by *P. irregulare* in hydroponic cucumber screening studies.
- the systemic acquired resistance inducers salicylic acid, sodium salicylate, BABA, potassium silicate, acibenzolar-S-methyl (Bion®) and methyl jasmonate were all ineffective at reducing disease caused by *P. irregulare* in hydroponic cucumbers. Some of these treatments were phytotoxic at the rates used.
- a commercial biological formulation 'Microplus™ (*Streptomyces lydicus*) and a non-pathogenic *Pythium* isolated from a Victorian vegetable farm (*P. oligandrum*) were ineffective at reducing disease caused by *P. irregulare* in hydroponic cucumbers.
- surfactants were phytotoxic to cucumber plants at the rates tested. Even the least phytotoxic treatments reduced plant growth below the level of the pathogen treatment alone and therefore were of no benefit for disease suppression.

3.1 Introduction

Soilless production with regulated supply of moisture and nutrients can result in more consistent yields and product quality than soil-based systems (Cockshull, 1985). The terms soilless or hydroponics have been defined as the production of crops isolated from the soil, either with or without a medium, and having their total water and nutrient requirements supplied by the system (Jensen, 1999; Hanger, 1993).

Australian production of hydroponic leafy vegetables is estimated to be 242 ha 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. For hydroponic leafy vegetables, production occurs mostly 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 at approximately 1ml/sec through

← Image previous page, chapter 3 coversheet – hydroponic cucumber plants in greenhouse trials exhibiting symptoms typical of *Fusarium* and *Pythium* diseases.

suspended plant roots in channels that are sloped to allow unused liquid to return by gravity 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.

The potential advantages of soilless systems may not be realised due to the prevalence of certain diseases (Jarvis, 1992). Diseases are particularly problematic in recirculated nutrient systems (Gold & Stanghellini, 1985; Menzies *et al*., 1998), particularly where farm and crop hygiene practices are poor or lacking (Jarvis, 1992). Further factors of soilless production systems can make them conducive to disease development once plant pathogens enter. They include: elevated nutrient temperatures in summer (Tesoriero & Cresswell, 1995; Alhussaen, 2006); stagnant water sitting around roots where there is poor drainage; and accumulation of excess mineral salts (Jarvis, 1992). Furthermore, low microbial populations and diversity found in soilless substrates and nutrient solutions are sometimes described as a 'biological vacuum' and one similar in this respect to fumigated soil. This is often associated with a lack of antagonistic microbes that 'buffer' the rhizosphere from plant pathogens (Postma *et al*., 2000). Soilless systems also favour the formation and dispersal of zoospores produced by several *Pythium* species (Stanghellini & Rasmussen, 1994).

In Australian hydroponic lettuce production, the water moulds, species of *Pythium* and *Phytophthora* and the fungus, *Thielaviopsis* have been reported to cause root diseases (Alhassaen, 2006; Tesoriero *et al*., 2007; Tesoriero *et al*., 2008; Hutton & Forsberg, 1991; O'Brien & Davis, 1994).

Many systemic chemical options for root diseases can lead to accumulation of undesirable residues when they are applied through the nutrient solution and this use pattern is therefore not likely to ever be registered in Australia or overseas. Microbial biocontrols offer a sound alternative and hydroponic NFT production is an excellent model system to study root rots and their suppression by beneficial microbes. Microbial biocontrol products consist of formulated fungal or bacterial inocula and they are becoming increasingly available to the Australian industry. However, to date they have only been assessed objectively for Australian hydroponic vegetables in lettuce production (Tesoriero *et al*., 2008). In that study, one commercial product containing a strain of the bacterium *Bacillus subtilis* consistently suppressed root disease expression to a level equivalent to uninfected control treatments. It appeared to reduce the colonisation of roots by the pathogen, *Phytophthora cryptogea*. In some trials it stimulated plant growth even in the absence of the pathogen. Similarly, certain biorational chemicals such as the plant defence activator, Bion® (Acibenzolar-S-methyl) have been shown to suppress disease development.

The role of silicon as a potential supplement in crop production has in the last twenty years been the focus of a large amount of research in plant biology (Menzies & Belanger, 1996; Epstein, 1999). There is substantial evidence that silicon affects plant development, increasing plant growth and yield in many species and that silicon can modulate plant resistant reactions to multiple pathogens (Epstein, 1999; Ma, 2004). Cherif *et al.* (1994) demonstrated that silicon application onto cucumber plants resulted in potentiation of chitinases, peroxidases and polyphenol oxidases when plants were later treated with *Pythium* spp. Recent research has suggested that silicon modulates a type of induced systemic resistance in wheat (Remus-Borel *et al.*, 2005), rice (Rodrigues *et al*., 2004) and pea (Dann & Muir, 2002), resulting in enhanced production of phytoalexins and pathogenesis-related (PR) proteins.

This study reports on preliminary trials to screen a range of novel products for management of *Pythium* and *Phytophthora* diseases of spinach, coriander (NSW) and *Pythium* diseases of cucumber (VIC) growing in hydroponic systems. In addition, an on-farm trial was conducted in NSW to evaluate chemical drenches for the management of *Fusarium* and *Pythium* diseases in greenhouse cucumber crops.

3.2 Rapid screening of novel and developing treatments – spinach and coriander (NSW)

3.2.1 Materials and Methods

Five experiments determined the relative pathogenicity of *Pythium* isolates and subsequent efficacy of microbial biocontrols and biorational chemicals. The experimental facility was a scaled-down version of commercial systems, consisting of 50 independent units, each with a 20L tank, submersible pump and plastic poly-pipe feeding nutrients to the top end of a sloping 3-metre length of PVC channel. Nutrients drained by gravity past suspended plant roots and back into each tank. Trials were designed as replicated blocks with each treatment randomised and consisting of continuously recirculated nutrients supplying 12 plants spaced along each NFT channel. Each treatment was replicated 4-10 times. An overview of the 5 experiments conducted over the project period is listed in Table 3.1.

Table 3.1. Pathogenicity and efficacy trials conducted on hydroponic vegetable crops

Trial Number	Dates	Treatments
1	Feb., 2009	Pathogenicity of *Pythium* & *Phytophthora* isolates to spinach and Pak choy
2	Mar., 2009	Pathogenicity of *Pythium* & *Phytophthora* isolates to coriander and two spinach cultivars
3	May, 2009	Efficacy of microbial biocontrols (*Bacillus subtilis* [Fulzyme Plus™ @2mL/L] and *Pythium oligandrum* [isolate 05/590]) to Pythium root rot (*P. aphanidermatum* [isolate 09/89]) in two spinach cultivars.
4	Aug., 2009	Efficacy of microbial biocontrols (*Bacillus subtilis* [Fulzyme Plus™ @2mL/L] and *Pythium oligandrum* [isolate 05/590]) and the plant defence activator Acibenzolar-S-methyl (Bion®) @2.5µL/L to Pythium root rot (*P. sulcatum* [isolate 03/822]) of coriander
5	Oct., 2009	Efficacy of microbial biocontrols (*Bacillus subtilis* [Fulzyme Plus™ @2mL/L]) plus phosphorous acid Phoscare® @2mL/L, and the plant defence activator Acibenzolar-S-methyl (Bion®) @2.5µL/L plus potassium silicate @650µL/L to Pythium root rot (*P. sulcatum* [isolate 03/822]) of coriander

Pythium isolates were initially obtained from diseased plant samples collected during surveys of commercial production. Roots were washed and plated to semi-selective agar media (potato carrot agar [PCA] amended with pimaricin [@5ppm] and rifampicin [@10ppm]). Plates were incubated at 25°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 initially identify species of *Pythium*. Molecular characterisation was performed by PCR amplification of the internal transcribed spacer (ITS) region of ribosomal RNA genes using primers ITS1 and ITS4, as described by White *et al.* (1990). Sequences of ITS regions were compared with GenBank databases and similarity analyses were used to place isolates into discrete taxa. *Phytophthora cryptogea* isolates were obtained in a previous study of leafy lettuce growing by hydroponics (Tesoriero *et al.*, 2008) and were used in the first two experiments to compare relative pathogenicity with *Pythium* isolates.

Seedlings were grown at a commercial nursery in plugs (105/tray) that were transplanted to NFT channels and allowed to establish prior to application of treatments. Seedlings in each plug were thinned to a consistent number for each plant species (2 per plug for Pak choy and 10 per plug for

coriander and spinach). Samples were taken from 3 plugs in each tray and roots were screened for background or confounding plant pathogens by plating 1 cm lengths to PCA.

Inocula for hydroponic trials were prepared from cultures grown on PCA at 25°C for 10-14 days. Cultures were then homogenised in distilled water and an equivalent of 1-2 plates were added to specified treatment tanks. Serial dilution of the inoculum suspensions and culturing to agar media was used to estimate pathogen concentrations using the most probable number technique (Cochran, 1950; Tesoriero 1989). Non-colonised PCA plates were homogenised to serve as negative control treatments. Inoculum rates were halved between experiments 4 and 5 in an effort to reduce disease pressure that may have masked treatment effects in the former trial.

Tanks were topped up with fresh nutrient as required. The plants were grown to maturity and harvested by cutting off leafy tops with scissors. They were then weighed to obtain mean fresh weights. Statistical analyses of data are detailed for each trial in the results section. Where the F-statistic was significant, pairwise differences significant at the 5 % level are indicated in the results using the superscript letter-based representation, where non-significant treatments have a common letter.

Roots samples were cultured as described above at the conclusion of trials to determine pathogen colonisation.

3.2.2 Results & Discussion

Trial 1

Data was analysed using ANOVA and treatment differences were tested using Fishers protected LSD test to compare treatment differences (LSD) test at the 5 % level. Mean fresh weights of 5 plugs of each plant species (Spinach and Pak Choy) are presented in Table 3.2. For spinach, all treatments except *P. aphanidermatum* had significantly lower average wet weights than the nil treatment. The *Phytophthora* isolates were strongly pathogenic with spinach yield reductions of almost 70 %. Interestingly, the *P. ultimum* isolate was more pathogenic (24 % yield reduction) than *P. aphanidermatum*. This latter species is generally considered to be a more aggressive pathogen and produces numerous zoospores in contrast to *P. ultimum* (which does not produce zoospores).

Table 3.2. Mean fresh weights (g) of Spinach and Pak Choy

Treatment (Isolate #)	Spinach	Pak Choy
Nil	94.9[c]	515.9 [bc]
Phytophthora cryptogea (#1805)	21.7[a]	499.2 [b]
Phytophthora cryptogea (#1756)	26.0 [a]	501.7 [b]
Pythium aphanidermatum (#297)	83.6[bc]	498.4 [b]
Pythium ultimum (#223)	72.0[b]	479.7 [b]
F-statistic	0	0.487
Average LSD (5 %)	14.67	39.27

Trial 2

Mean fresh weights of 6 plugs of coriander cv. *Santo*, 4 plugs of spinach cv. *Crocodile* and 2 plugs of spinach cv. *Winter Giant* are presented in Table 3.3. The average coriander fresh weight was significantly lower for *Pythium sulcatum* isolate 03/822 than for the remaining treatments. For Spinach, there was variance heterogeneity, so significant pairwise treatment differences were determined using an LSD specifically calculated for each pairwise difference in turn. The treatments with the lowest fresh weights also had the largest spread, implying that they not only decreased growth but also increased variation in growth. Two residual variances were therefore fitted, one for these treatments and one for the remaining six treatments, to allow for this variance. An analysis was performed in ASReml, a linear mixed model package, which allowed separate residual variances to be fitted for each

set of treatments. For both cultivars, average fresh weights of *Phytophthora cryptogea* isolate 08/174-1 and *Pythium sulcatum* isolate 03/822 treatments were significantly lower than the Nil treatment.

Table 3.3. Mean fresh weights (g) for pathogen treatments

Treatment (isolate #)	Coriander	Spinach cv. Crocodile	Spinach cv. Winter Giant
Nil	781.5[b]	130.8[bc]	273.0[b]
Phytophthora cryptogea (08/174-1)	795.3[b]	39.8[a]	127.2[a]
Phytophthora cryptogea (08/581)	792.0[b]	133.9[bc]	252.2[b]
Pythium coloratum (07/1122)	760.9[b]	124.4[b]	270.2[b]
Pythium aphanidermatum (09/89)	779.3[b]	62.6[a]	167.8[a]
Pythium sulcatum (03/822)	623.0[a]	151.9[c]	321.6[c]
Pythium ultimum (04/710)	786.3[b]	136.1[bc]	270.1[b]
Pythium coloratum (07/1042-3)	780.3[b]	124.1[b]	296.3[bc]
Average LSD (5 %)	72.09		
F-statistic	4e-04	6.2e-09	5.9e-08

Trial 3

Presence or absence of the pathogen treatment, *Pythium aphanidermatum* isolate 09/89, had no significant effect on mean fresh weights of either spinach cultivar. This may have been due to the cooler temperatures experienced during this trial. The mean nutrient temperature was 20°C and there were only 8 of the 50 days during which the trial was conducted that the temperature reached 30°C. *Pythium aphanidermatum* is known to have a requirement for temperatures above 30°C to exhibit pathogenicity. There was no significant statistical interaction between coriander cultivars and chemical/biological treatments, therefore fresh weights have been averaged in Table 3.4. Note that there was a significant growth stimulation effect of *Bacillus subtilis* (Fulzyme Plus™) compared with other treatments.

Table 3.4. Mean fresh weights (g) of Spinach cvs *Crocodile* & *Winter Giant*

Treatment (isolate #)	Spinach mean fresh weight (g)
Nil	272.7[a]
Agral®	282.0[a]
Pythium oligandrum (isolate 05/590)	276.9[a]
Fulzyme Plus™	329.6[b]
LSD (5 %)	33.2

Trial 4

Pythium sulcatum isolate 03/822 significantly reduced fresh weights compared with nil pathogen treatments. However the analysis showed that there was no significant interaction between the pathogen and biological treatments; that is the biological/chemical controls did not reduce disease expression in the presence of the pathogen. When the data was analysed separately for biological/chemical treatments and pathogen, there was a significant growth stimulation effect of *Bacillus subtilis* (Fulzyme Plus™), and *P. oligandrum* compared with Bion® and Nil treatments (Table 3.5).

Table 3.5. Mean fresh weights (g) for coriander cv. Santo in Trial 4

Treatment	Fresh weight (g)	Transformed fresh weights ($x^{0.8}$)
Nil	867.1	1.6966
P. sulcatum	12.0	1.1913
Standard error		0.0177
Nil	390.0	1.3947[a]
Bion®	277.1	1.3961[a]
Fulzyme Plus™	628.0	1.5097[b]
P. oligandrum	463.2	1.4753[b]
LSD (5 %)		0.0576

Trial 5

An analysis was performed in ASReml and a square root transformation was required to stabilise the residuals. Overall, Bion® + Silicon reduced yield in the absence of the pathogen, but increased it with the pathogen (Table 3.6). This suggests phytotoxicity at this application rate but also some disease suppression. Future trials could explore lower concentrations of Bion®. In contrast, the *Bacillus subtilis* (Fulzyme Plus™) plus Phosphorous acid (Phoscare®) treatment had no effect on fresh weights in the absence of the pathogen and very significantly increased it with the pathogen (Table 3.6). Observations made during the trial were that this treatment suppressed disease symptoms up until a fortnight prior to harvest. Plants appeared to have similar growth rates to those of the uninfected control treatments which had white healthy roots. After that time roots began to progressively display typical brown root rot symptoms and older leaves on many plants began to yellow and wilt. A second application of these products may therefore be useful for sustained disease suppression and would be worthy of future experiments.

Table 3.6. Mean fresh weights (g) for coriander cv. Santo in Trial 5

Pathogen	Treatment	Fresh weight (g)	Transformed fresh weight (√)
Nil	Nil	713.1	27.23[a]
	Bion® + Silicon	556.5	23.58[b]
	Fulzyme Plus™ + Phoscare®	772.8	27.79[a]
	Bion® + Silicon + Fulzyme Plus™ + Phoscare®	480.7	21.89[c]
P. sulcatum	Nil	73.1	8.53[f]
	Bion® + Silicon	121.7	10.89[e]
	Fulzyme Plus™ + Phoscare®	220.3	14.77[d]
	Bion® + Silicon + Fulzyme Plus™ + Phoscare®	224.4	14.97[d]
LSD (5 %)			1.46

3.2.3 Conclusions

Plant pathogenic *Pythium* and *Phytophthora* isolates were confirmed causing root rot diseases of spinach and coriander growing in recirculated hydroponic systems. *Pythium aphanidermatum* (09/89) and *Phytophthora cryptogea* (08/174-1) were the most aggressive isolates tested on spinach while *Pythium sulcatum* was the most aggressive species to coriander. Unfortunately the single trial (#3) assessing the efficacy of microbial biocontrols and biorational chemical treatments for root rot of spinach caused by *P. aphanidermatum* failed, most likely due to cooler nutrient temperatures experienced during the trial period in contrast to the previous two pathogenicity trials. Attempts to assess similar treatments for Pythium root rot of coriander were more successful with respect to reproducing disease symptoms using *P. sulcatum* inoculum. In fact, it was concluded that the inoculum concentration may have swamped any efficacy effects of treatments in trial #4 and it was subsequently halved in the following trial (#5) to better effect where a single treatment of *Bacillus subtilis* (Fulzyme Plus™) plus Phosphorous acid (Phoscare®) significantly suppressed disease symptoms. A second application of these products, perhaps 2-3 weeks after the initial treatment, should be assessed in further trials to assess for more sustained disease suppression. Plant growth stimulation was demonstrated with the *Bacillus subtilis* treatments in Trials 3 & 4 and to a lesser extent with the *P. oligandrum* isolate in trial 4.

3.3 Rapid screening of novel and developing treatments – cucumber (VIC)

3.3.1 General Materials and Methods

Plant material

Cucumber (Lebanse Melen F1, South Pacific Seeds) seed was germinated in seed raising mix and seedlings were transplanted into LP50 hydroponic pots filled with perlite. Plants were grown in aerated hydroponics in 2.5 L plastic tubs with 4 plants per tub. A one part nutrient mixture ('Greendream' Flairform WA) was added to the tubs at a rate of 5 mL/L.

Treatment application

Treatments were applied three days pre-inoculation and plants were exposed to the treatment for the duration of the experiment. Unless otherwise stated (see below), treatments were prepared in the nutrient mixture.

Inoculation

Pythium irregulare was grown on V8 agar at 20ºC for 7-14 days. The plates were then blended with deionised water (100 mL deionised water per plate) in a domestic blender for 30 seconds and 100 mL of the resulting slurry used to inoculate the hydroponic tubs containing cucumber plants three days after treatment application. V8 agar plates not inoculated with *P. irregulare* were blended with deionised water and added to the tubs of the control treatment.

Disease assessment

Plants were harvested 16-17 days after inoculation. The perlite was washed from the roots and plants were blotted dry. Fresh weights of the entire plant, root weight and shoot weight were recorded.

3.3.2 Systemic acquired resistance and biological control in cucumber

Materials and methods

Three trials were conducted to evaluate a range of SAR inducers and biological controls for control of diseases caused by *Pythium* spp. in hydroponic cucumber (Table 3.7).

Salicylic acid solution preparation
A 10 mM stock Salicylic acid (SA) solution was prepared by dissolving SA in hot deionised water with stirring. The stock solution was adjusted to pH 7 with NaOH and then diluted to 200 µM in nutrient solution.

Chitin and Chitosan preparation
Chitosan from crab shells, practical grade (Aldrich, NSW) and a liquid chitin (Ellis and Associates, Vic) were prepared according to the following protocols. Chitosan was dissolved in 0.3 % acetic acid at the rate of 1 g chitosan/100mL while stirring and then adjusted to pH 6.2 with NaOH. The chitosan stock was then diluted to 0.2 g/L with hydroponic nutrient solution.

Methyl Jasmonate preparation
Methyl Jasmonate (MeJ 95 %) (Aldrich NSW) – stock solution was prepared to give 314 mM MeJ in ethanol. Diluted to final concentration of 100 uM in the hydroponic nutrient solution.

Table 3.7. Treatment details for SAR and biological control trials conducted in experimental hydroponic units to evaluate products for their ability to control diseases caused by *Pythium* spp. in cucumber.

Treatment	Supplier	Rate	Proposed mode of action	Trial
β - aminobuturic acid (BABA)	Sigma NSW	50 mg/L	SAR inducer	1
Silika Majic™ (20 % soluble silca)	Flairform WA	160 µL/L Adjusted to pH 6.7 with HCl	Structural and/or SAR inducer	1
Liquid chitin	Ellis and associates, Vic	0.015 % 0.003 %	SAR inducer	1 2 & 3
Microplus™ (*Streptomyces lydicus)*	Organic Farming Systems, WA	0.02 g/L	Biological	2
Sodium salicylate	Aldrich NSW	200 µM	SAR inducer	2
Bion® 50 WG (acibenzolar-S-methyl)	Syngenta	0.028 g/L (1.4 mg/L a.i.)	SAR inducer	2
Chitosan	Aldrich, NSW	0.2 g/L	SAR inducer	2 & 3
Pythium oligandrum	Des Auer, DPI Vic (ex. Parsley)	1 V8 plate per 100 mL	Biological	3
Methyl jasmonate	Sigma, NSW	100 µM	SAR inducer	3
Salicylic acid	BDH, England	200 µM	SAR inducer	3

Results

Inoculated control seedlings had poor root growth, wilted and were stunted. Effective treatments reduced wilting and improved seedling vigour (measured as root, shoot and total plant weight). Chitin and chitosan consistently resulted in higher plant, shoot and root weight than the inoculated control. These differences were often, although not always statistically significant (Tables 3.8-3.10). None of the other treatments resulted in improved disease control sufficient to cause a statistically significant increase in plant, shoot or root weight. Bion® was highly phytotoxic at 28 mg/L and Bion® treated plants were all dead within 10 days of treatment (data not shown). Likewise methyl jasmonate appeared to be phytotoxic at the rate used as the mean plant weight at assessment was significantly lower than the inoculated control (Table 3.10).

Table 3.8. Trial 1 - The effect of treatments of the growth of cucumber seedlings in hydroponic solution when inoculated with *P. irregulare.*

Treatment	Shoot weight (g)	Root weight (g)	Total plant weight (g)
Control (non-inoculated)	4.7 a	1.1 a	5.8 a
Control (inoculated)	1.7 c	0.2 c	1.8 c
Liquid chitin+inoc	2.1 b	0.4 b	2.5 b
BABA+inoc	1.6 c	0.2 c	1.8 c
Silika Majic™+inoc	1.5 c	0.2 c	1.7 c
LSD (5 %)	0.4693	0.1519	0.5727

Table 3.9. Trial 2 – The effect of treatments of the growth of cucumber seedlings in hydroponic solution when inoculated with *P. irregulare.*

Treatment	Shoot weight (g)	Root weight (g)	Total plant weight (g)
Control (non-inoculated)	15.5 a	6.2 a	21.7 a
Control (inoculated)	3.7 c	0.6 c	4.3 bc
Chitosan	8.1 b	2.1 b	10.2 b
Liquid chitin	6.7 b	1.2 bc	7.9 b
NaSalicylate	2.9 c	0.4 c	3.3 c
Microplus™	2.9 c	0.4 c	3.3 c
LSD (5 %)	2.834	1.088	3.747

Table 3.10. Trial 3 – The effect of treatments of the growth of cucumber seedlings in hydroponic solution when inoculated with *P. irregulare.*

Treatment	Shoot weight (g)	Root weight (g)	Total plant weight (g)
Control (non-inoculated)	32.4 a	9.6 a	42.1 a
Control (inoculated)	10.2 c	1.8 bc	11.9 cd
Liquid chitin	19.7 b	3.9 b	23.6 b
Chitosan	16.3 bc	3.0 b	19.3 bc
Pythium oligandrum	14.6 bc	2.8 b	17.4 bc
Salicylic acid	13.4 bc	2.6 bc	16.1 bc
Methyl jasmonate	3.4 c	0.5 c	3.9 d
LSD (5 %)	6.893	2.226	8.93

3.3.3 Surfactants for managing *Pythium* diseases in cucumber

Materials and methods

Two trials were conducted to investigate the effects of surfactants on Pythium disease of cucumber in hydroponics. Both trials were inoculated with *P. aphanidermatum,*although trial 1 also had a treatment inoculated with *P.irregulare* to compare its pathogenicity with that of *P. aphanidermatum*. Treatments (Table 3.11) and inoculations were performed as described previously (3.3.1).

Table 3.11. Surfactants used in hydroponic screening studies

Surfactant	Type	Supplier	Rate
Du-wett®	Organosilicone	Elliot chemicals (NZ)	0.5 mL/L
Spraymate LI700®	Soyal Phospholipid	Nufarm (Vic)	0.4 – 5 mL/L
Terwet 1300®	Polyalkylene glycol	Huntsman (Australia)	5 mL/L
Hortiwett®	Alkylarylpolyglycol ether	Nipro (Qld)	0.01 - 0.1 mL/L

Results

In both surfactant trials, all of the surfactants treatments resulted in reduced growth and/or phytotoxicity relative to both *P. aphanidermatum* inoculated and uninoculated control treatments (Figs 3.1 & 3.3).

In Surfactant trial 1, there was no significant difference between plant weights in the inoculated (*P. aphanidermatum*) and non-inoculated control treatments while the *P. irregulare* inoculated treatment had a significantly lower plant weight than both the control treatments. (Fig. 3.1). All of the adjuvant treatments had plant weights significantly lower than the non-inoculatated control. This appears to be due to phytotoxicity as inoculation with *P. aphanidermatum* alone did not result in a decrease plant weight at assessment. The degree of phytotoxicity varied ranging from complete collapse of plants within 24 h of treatment (Du-wett® and the higher rate of Spraymate LI700®) to no visible symptoms other than decreased plant weight at assessment (Terwet® 1300) (Fig. 3.1). Preliminary work showed Spraymate LI700® to be highly phytotoxic at 5 mL/L with plant stems being completely dessicated within 24 h (Fig. 3.2). A lower rate 0.4 mL/L was subsequently tested but symptoms of phytotoxicity were still evident although the plants were not killed. Du-wett® was highly phytotoxic with only 4 out of 16 plants surviving until assessment (16 days post treatment). Hortiwett® treated plants grew very poorly but were not killed by the treatment. As even the least phytotoxic treatments reduced plant growth below the level of the pathogen treatment alone, therefore they were of no benefit for disease suppression.

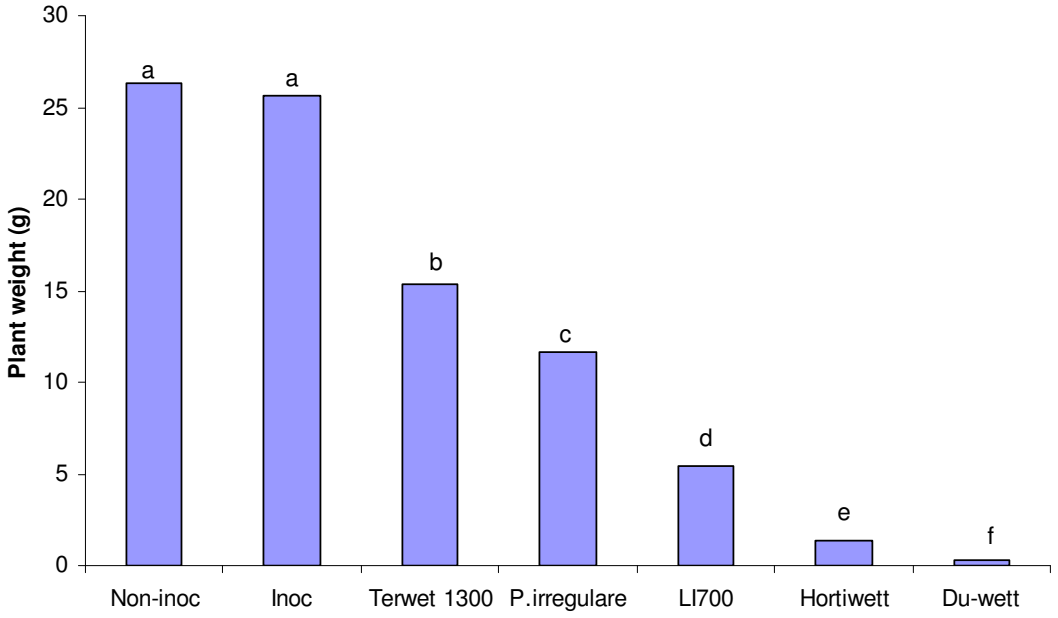


Figure 3.1. Surfactant trial 1 - Total plant weights of hydroponically grown cucumbers16 days post-inoculation with *P. aphanidermatum*. Non-inoc=non-inoculated control, Inoc=inoculated control (*P. aphanidermatum*). Different letters above the bars indicate values are significantly different at the 5 % level

Figure 3.2. Phytotoxicity symptoms on cucumber plants, a) desiccated stems of plants treated with 5 mL/L LI700® and b) collapsed stems of two plants treated with 0.5 mL/L Du-wett®, (24 hours post-treatment) in Surfactant trial 1.

In Surfactant trial 2, there was a significant difference in plant weight between the *P. aphanidermatum* inoculated and uninoculated control treatments. Hortiwett® still showed considerable phytotoxicity at 0.01 mL/L ($1/10^{th}$ of the rate used in Surfactant trial 1)(Figure 3.3) and had a significantly lower plant weight at assessment than the *P. aphanidermatum* inoculated only treatment.

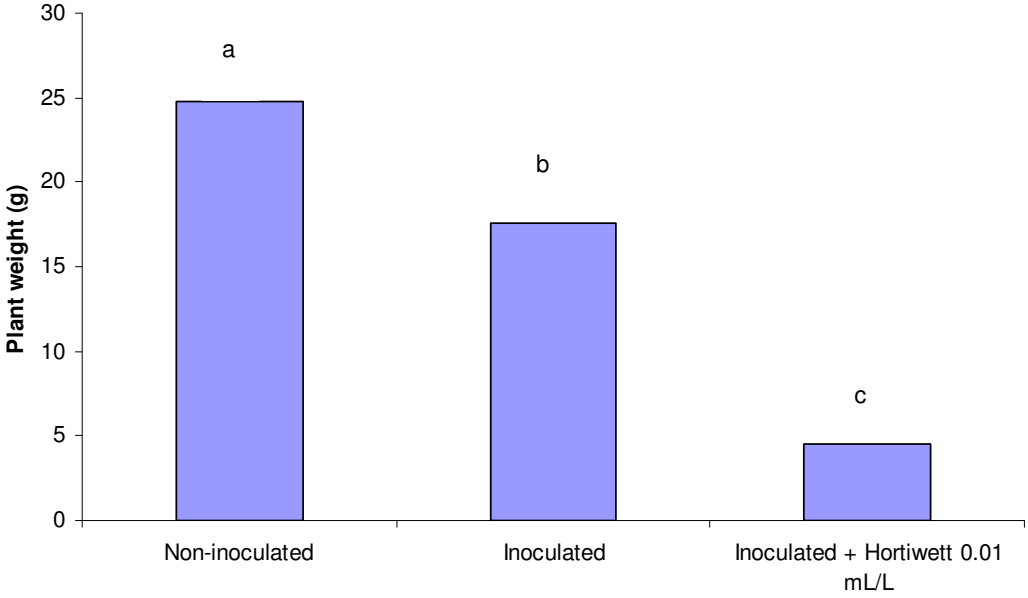


Figure 3.3. Surfactant trial 2 – Total plant weights of hydroponicaly grown cucumbers 16 days post-inoculation with *P. aphanidermatum*. Different letters above the bars indicate values are significantly different at the 5 % level.

3.3.4 Conclusions

Both the liquid chitin formulation and chitosan treatments consistently reduced the severity of disease caused by *P. irregulare*. Plant weights for these treatments 16-17 days after inoculation were up to double the inoculated control treatment. The mode of action of the chitin and chitosan treatments used in these trials is not known but chitosans are known to induce plant defence responses and also to have direct toxicity to pathogenic organisms. Chitosan has been shown to strongly inhibit zoospore motility when tested against grapevine downy mildew (Riches and Holmes 2005). The test organism used in this study, *P.irregulare*, was chosen as it produced the most consistent and severe symptoms in preliminary trials. However, this species does not produce zoospores. It is possbile that the liquid chitin and chitosan treatments may be even more effective against zoospore producing oomycetes pathogens than they were against *P. irregulare* in this study. Chitin and chitosan are polymeric materials derived from crustacean exoskeletons and their non-toxic nature would be an advantage if they were to be developed as crop protection chemicals.

The chemical treatments: salicylic acid, sodium salicylate, BABA, potassium silicate, acibenzolar-S-methyl (Bion®) and the biological treatments: Microplus™ and *P. oligandrum* were all ineffective at reducing disease on hydroponically grown cucumber. In the cases of Bion® and Methyl Jasmonate, these treatments were highly toxic to the cucumber plants.

In the surfactant trials, *P. aphanidermatum* (a zoospore producing *Pythium*) was used as a test organism instead of the more pathogenic non-zoospore producing *P. irregulare*, as the mode of action of surfactant products is known to be zoospore lysis (Stanghellini and Miller, 1997). However, disease caused by *P. aphanidermatum* was inconsistent, possibly due to lower than optimal temperatures in the hydroponic media. All of the surfactants tested resulted in reduced growth relative to both inoculated and uninoculated controls. The degree of phytotoxicity varied ranging from complete collapse of plants within 24 h of treatment (Du-wett® and the higher rate of LI700®) to no visible symptoms other than decreased plant weight at assessment (Terwet 1300®). However, even the least phytotoxic treatments reduced plant growth below the level of the pathogen treatment alone and therefore were of no benefit for disease suppression.

3.4 On-farm evaluation of chemical drenches to manage *Fusarium* and *Pythium* diseases in cucumber (NSW)

On-farm trials were conducted to assess the effect of chemical drenching on disease development, plant survival and cucumber yield. In the second trial the effects of improved hygiene and on-farm sanitation combined with chemical drenching were also assessed. Data was collected with the long-term view of applying for permits in collaboration with AgAware Consulting and HAL.

3.4.1 Materials and methods

Trial 1

Four rows within a commercial cucumber crop were used for the trial. Cucumber plants in cocopeat bags were treated by drenching with the chemicals Octave®, Amistar® and Flint® beginning when the plants were four weeks old and every two weeks thereafter. Two different rates of Octave® and Amistar® were used. The trial was established as a randomised block with 4 blocks (rows) and 5 treatments giving 4 replicates per treatment. Each replicate consisted of 10 bags, each bag containing 3 plants. Three cocopeat bags with cucumber plants were left untreated as a buffer at the end of each row.

Cucumber fruit of a marketable size was picked every two to three days, from each treatment block. Fruit numbers were counted and recorded. Disease levels were assessed on a weekly basis, with plants rated from 0-3, with 0 indicating no disease; 1 indicating low disease symptoms ie. water soaking at the base of the cucumber stem; 2 indicating *Fusarium* sporodocia lesions on the stem; and 3 indicating a fatal wilt of the cucumber plant and/or plant death.

The plants were grown under commercial conditions. Fertiliser was applied through drippers and appropriate disease control methods for other diseases were undertaken e.g. applying a chemical spray for powdery mildew.

Trial 2

Six rows within a commercial cucumber crop were used for the trial. These were split into two sub-experiments with half the trial area cleaned by the grower as he would typically clean the greenhouse between crops. This included the replacement of drippers and weed matting. The other half of the trial was cleaned and sanitised using improved hygiene practices. These included thorough cleaning of all surfaces within the greenhouse with a high pressure water gurney mixed with Virkon™ detergent; scrubbing of floor and drainage channels to remove all caked-on soil and plant debris; weeds cut using a whipper snipper before being sprayed out using a herbicide; replacement of weed matting and drippers. Cucumber seedlings in both halves of the experiment were established in new cocopeat bags and were treated by drenching within one day of being planted into the greenhouse and every two weeks thereafter. The treatments used in the trial were two different rates of Octave® and Amistar® and a mixture of Bion®, potassium silicate, Fulzyme™Plus (*B. subtilis*), Tri-D25 (*Trichoderma harzianum* & *T. Koningii*), and two different strains of putative *Psuedomonas fluorescens* biocontrol bacteria isolated during the project.

The trial was a factorial design to determine the effect of drench treatments and improved hygiene on disease development, plant survival and cucumber yield and to establish any interaction between these factors. Each half of the trial was established as a randomised block with 3 blocks (rows) and 5 treatments giving 3 replicates per treatment. Six cocopeat bags with cucumber plants were left as untreated buffers on either side of the treated plants.

Trial maintenance, and the harvesting and assessment of cucumbers was as described for trial 1 (above).

3.4.2 Results and Discussion

Trial 1

Statistically there was no difference in either the level of disease observed or the numbers of fruit picked that could be contributed to any of the chemical treatments. Approximately 11, 400 cucumbers were picked within the trial with an approximate weight of 1 700kg. There was a trend for less disease with the Amistar® high treatment (Fig. 3.4), but this was not significant at the 95 % level.

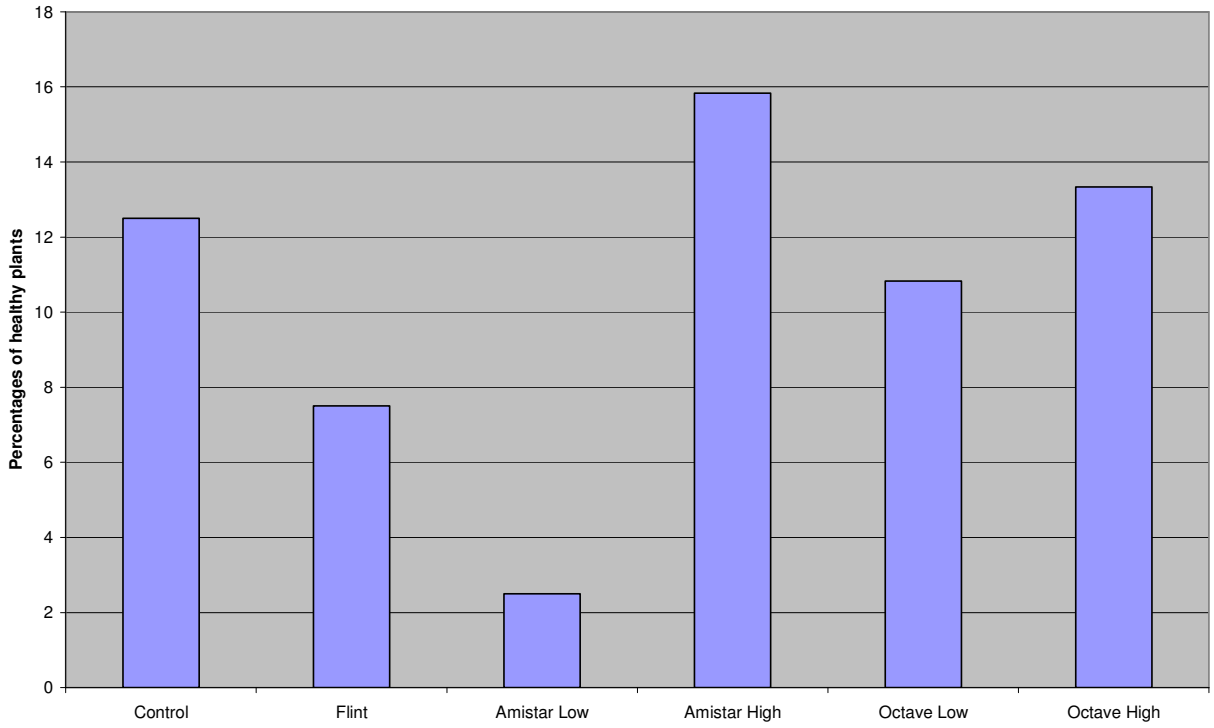


Figure 3.4. The effect of drenching on the numbers of healthy cucumber plants two months into the commercial farm experiment.

Trial 2

Statistically there was no difference in either the level of disease observed or the numbers of fruit picked that could be contributed to any of the treatments. Approximately 27, 970 cucumbers were picked within the trial with an approximate weight of 4 200kg. There was a trend for less disease with the Amistar® high, Octave® high and the mixture (biologicals, potassium silicate and Bion®) treatments (Fig 3.5) but this was not significant at the 95 % level.

Elsewhere within the greenhouse, including in the immediate vicinity of the trial area the grower had reused cocopeat bags. Disease loss was nearly 100 % in these areas. Due to the movement of airborne inoculum from these areas into the experimental area disease progress within the trial was heavily biased towards the side of the greenhouse where the grower had reused cocopeat bags. Since inoculum was not applied and only entered the trial area naturally, disease levels within and between rows was not consistent and a large 'edge effect' was observed. Therefore differences could not be drawn between the areas cleaned by the farmer and those cleaned using the modified hygiene practices. This highlights the contribution of reused cocopeat bags to the build up of inoculum in the greenhouse and subsequent crop losses.

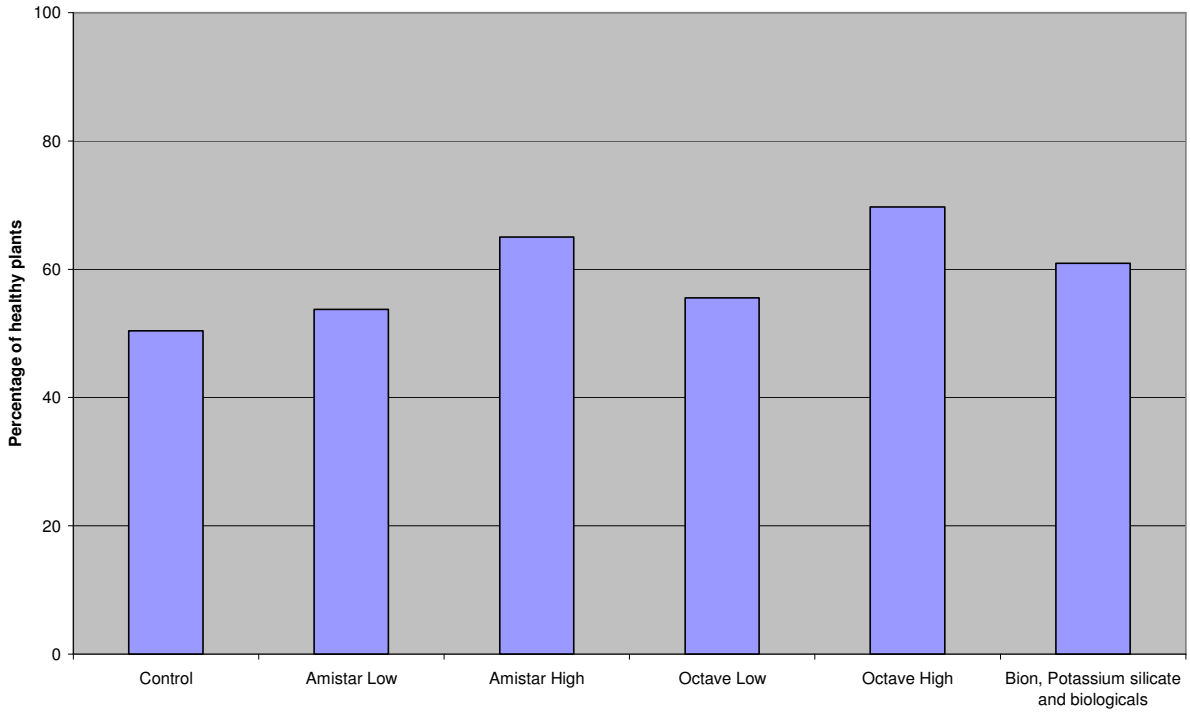


Figure 3.5. The effect of chemical drenching on the numbers of healthy cucumber plants at the conclusion of the second commercial scale on-farm experiment

3.5 Acknowledgements

The authors would like to thank I&I, NSW technical staff Jon Collins, Tehgan Crowe and Kulan Turton for assistance in conducting these trials. Thanks to commercial hydroponic growers in NSW and South Australia for providing diseased plant material for pathogen isolation and Leppington Speedy Seedlings for providing healthy seedlings for disease trials. We thank Joe Boustani, commercial hydroponic grower for allowing us to conduct on-farm trials on his property and for assistance with the establishment and maintenance of the trials. Zadco for Quality Gro Pty Ltd provided Fulzyme Plus™ and Phoscare® and Syngenta Ltd provided Bion® for evaluation.

3.6 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.

Cherif M, Asselin A and Belanger RR (1994) Defence responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. *Phytopathology* 84: 236-242.

Cochran, W.G., 1950. Estimation of bacterial densities by means of the "most probable number". *Biometrics*, 6:105-116.

Cockshull, K.E. 1985. Greenhouse climate and crop response. *ACTA Horticulturae*, 13:464-466.

Dann EK and Muir S (2002) Peas grown in media with elevated plant-available silicon levels have higher activities of chitinase and beta-1,3-glucanase, are less susceptible to a fungal leaf spot pathogen and accumulate more foliar silicon. *Australasian Plant Pathology* 31: 9-13.

Epstein E (1999) Silicon. *Annual Review of Plant Physiology and Molecular Biology* 50: 641-664.

Gold, S.E. and Stanghellini, M.E. 1985. Effects of temperature on *Pythium* root rot of spinach grown under hydroponic conditions. *Phytopathology*, 75:333-337.

Hanger, B. 1993. Hydroponics: The world, Australia and South Pacific Islands scene. In: *Commercial Hydroponics in Australasia, a Guide for growers*. Pro-Set Pty Ltd.

Hutton, D.G. and Forsberg, L. (1991). Phytophthora root rot in hydroponically grown lettuce. *Australasian Plant Pathology,* 20:76-79.

Jarvis, W.R. 1992. *Managing diseases in greenhouse crops*. APS Press, St. Paul, USA 288pp.

Jensen, M.H. 1999. Hydroponics Worldwide. In: *Proceedings of the international symposium on growing media and hydroponics*, Ontario, Canada 19-26 May 1997, (Ed. A.P. Papadopoulos). *Acta Horticulturae*, 481:719-729.

Ma JF (2004) Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science and Plant Nutrition* 50: 11-18.

Menzies JG, Belanger RR (1996) Recent advances in cultural management of diseases of greenhouse crops. *Canadian Journal of Plant Pathology* **18,** 186-193.

Menzies, J., Ehret, D., Barr, C. and Koch, C. 1998. *Pythium* spp. Isolated from healthy roots of cucumber plants growing in hydroponic culture. *Canadian Journal of Plant Pathology*, 20: 125.

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 (1981). Monograph of the genus *Pythium*. *Studies in Mycology* 21:242pp.

Postma, J., Willemsen-de Klein, M., and van Elsas, J. 2000. Effect of indigenous microflora on the development of root and crown rot caused by *Pythium aphanidermatum* in cucumber grown on rockwool. *Phytopathology,* 90:125-133.

Remus-Borel W, Menzies JG and Belanger RR (2005) Silicon induces antifungal compounds in powdery mildew-infected wheat. *Physiological and Molecular Plant Pathology* 66: 108-115.

Riches D. and Holmes R. (2005) Control of downy mildew of grapevines by boosting their natural defence system Final Report to GWRDC Project DNR 02/05.

Rodrigues FA, McNally DJ, Datnoff LE, Jones JB, Labbe C, Benhamou N, Menzies JG and Belanger RR (2004) Silicon enhances the accumulation of diterpenoid phytoalexins in rice: A potential mechanism for blast resistance. *Phytopathology* 94: 177-183.

Stanghellini, M.E. and Miller R.M. (1997) Biosurfactants Their identity and potential efficacy in the biological control of zoosporic plant pathogens. *Plant Disease* 81: 4-12,

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

Tesoriero, L. 1989. *Pythium* species associated with wheat in Central-western NSW. M.Sc. (Agr.) Thesis, University of Sydney, 173pp.

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.

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols : A guide to Methods and Applications* (ed. MA Innis, DH Gelfand, JJ Sninsky & TJ White), pp. 315-322. Academic Press : San Diego, U.S.A.

Grafting and *Fusarium* wilt of snake bean

4. Grafting and *Fusarium* wilt of snake bean

Researchers: Barry Condé, Mark Traynor, Dr Lucy Tran-Nguyen and Sean Bithell (Plant Industries, Department of Resources, Northern Territory Government).

The effectiveness of grafting on to the resistant Iron cowpea root stock as a means of controlling *Fusarium* wilt was evaluated and demonstrated to snake bean growers through a series of large scale on-farm demonstration trials and training events. This work has demonstrated that:

- grafting is advantageous in all situations where *Fusarium* wilt of snake bean occurs.
- it is likely to be economically advantageous to use grafted plants over seedlings where infection levels are moderate to severe. Under such conditions grafted plants are likely to yield twice as much as ungrafted seedlings.
- under low disease pressure, the resulting yield increases will not offset the extra costs (mainly labour) of producing grafted plants.

Previous studies demonstrated the existence of 3 *Fusarium oxysporum* f.sp. *tracheiphilum* (Fot) strains (based on colour in culture), which are pathogenic to snakebean in the Northern Territory. In this present study race differential work demonstrated that the Fot 'plum' strain is similar to, but may be slightly different from known race 2, while the 'white' and 'pink' Fot strains are different to the four known races of Fot. Several genetic marker techniques were used to further characterise *Fusarium oxysporum* f.sp. *tracheiphilum* isolates ('white', 'pink' and 'plum') from the Northern Territory (NT). Based upon 25 isolates in total, there was no direct evidence that any were genetically identical to the four known *Fot* races. This implies that the three NT isolates may be different races to those found previously. It is therefore important that any resistant varieties or rootstocks imported from overseas for use in the Northern Territory are screened against the three local isolates. The genetic analyses indicated that the 'white' and 'pink' isolates were closely related compared with the 'plum' and *Fot* races 1 to 4. The 'plum' isolate may be a close relative of the *Fot* race 2. There was no genetic diversity among the isolates within the individual colour isolates across different locations.

4.1 Introduction

Snake beans and Fusarium wilt

Snake bean (*Vigna unguiculata* subsp. *sesquipedalis*) is a major crop grown by the Asian vegetable industry in Northern Australia. The main growers are Vietnamese. The snake bean industry throughout Australia is based on the Green Pod Kaohsiung (GPK) variety, a dark tipped variety preferred by the markets. GPK was introduced into Australia in 1990 through the Post Entry Quarantine in Darwin. Snake beans are grown in SE Queensland and NSW during the hot summer months. The Northern Territory grows and supplies snake beans during its dry season (May – Oct) so the supply from the NT does not clash with the supply from SE Queensland (coastal areas around Brisbane) and NSW (Sydney Basin) (Greg Owens, NT DPIFM pers. com).

The crop has encountered many problems, such as various leaf diseases, root knot nematode, bean fly, bird damage, two spotted mites and *Pythium* base rot (associated with water logging). Most of these problems have appropriate management treatments, however in 1999 growers began to experience a new problem, *Fusarium* wilt. This is one of several conditions that the Vietnamese call 'early die' along with root knot nematode and *Pythium* base rot. The distinguishing features of *Fusarium* wilt is that plants die more slowly than those affected by *Pythium*, but faster than those affected by root knot nematode. Most importantly, the internal water conducting tissues in the base of the plant are dark in colour and leaves are yellow. Snake bean *Fusarium* wilt is caused by the fungus, *Fusarium oxysporum* f.sp. *tracheiphilum* (E.F. Smith) Syn. & Hans. (abbreviated as Fot). Four races of this fungus are known in the world and all occur in the USA (Armstrong and Armstrong 1980; Smith *et al.* 1999), all of which also attack cowpea. Erwin Smith first described *Fusarium* wilt diseases of cowpea in 1899 (Smith 1899). This forma specialis is defined by its ability to infect and cause a vascular wilt of plants of the species *Vigna unguiculata* (Booth 1971). Race 1 also causes disease in soybeans (Armstrong and Armstrong 1950) and chrysanthemums (Armstrong and Armstrong 1965).

↩ Image previous page, chapter 4 coversheet - production of grafted snake bean plants.

Fot has worldwide distribution, occurring in many places where cowpeas are grown. This includes many states in the USA (California, Georgia, Mississippi, North Carolina and South Carolina), India, China, and Japan in Asia, Hungary, Italy and Russia in Europe and Egypt, Nigeria and Tanzania in Africa (Cabi Compendium). Fot is also reported from Brazil in South America (Assunção et al. 2003; Rodriguez & Menezes 2005). There is an active breeding program in California, but not in Mississippi or Georgia where the disease is no longer of major concern. It is possible that the resistant varieties bred by Hare (1953) and others may have helped to alleviate the disease problem in Georgia and Mississippi. Fusarium wilt has been reported in snake beans from China (Fang and Hwang 1987) and Taiwan (Yi-Sheng Lin pers. com 1999; Ha and Huang 2007). Singh (1980) listed Fusarium wilt as a disease of snake beans in peninsular Malaysia and as a serious and economically important snake bean disease in Sabah. Fusarium wilt is listed as a disease of snake beans in Brunei (Peregrine and Ahmad 1982). Snake bean Fusarium wilt is serious in Sarawak (William Teo and Lily Eng, pers. comm. 2003, 2009) although, it is not known to occur in Indonesia (Siti Subandiyah and Arif Wibowo, Gadjah Mada University, pers. comm.). China and Taiwan have both investigated the use of resistant varieties. However, this may not have solved the problem, because Taiwan has since requested the Iron cultivar from the Northern Territory [as a result of the Northern Territory Agnote on grafting snake beans (Tony Gilmore, Australian Pasture Seeds, pers. comm.)].

The Fusarium wilt pathogen

F. oxysporum isolates of a given forma specialis are not usually distinguishable morphologically, and subsequent subdivision into physiological races is based upon the ability to cause disease on a set of differential cultivars (Gordon 1965). Four physiological races are described, all of which occur in USA (Smith *et al.* 1999). Armstrong and Armstrong in 1950 described two races of Fot. They also found Race 1 to cause wilt in soybean (Armstrong and Armstrong 1950) and chrysanthemum (Armstrong and Armstrong 1965). Hare (1953) reported a third race causing wilt of cowpeas in Mississippi, with the cultivar Arlington serving as the differential host for Race 3. Arlington was infected by race 3, but not by race 1 and 2. Hare (1953) developed the differential varieties, Groit, Chinese Red and Arlington which were subsequently used by Toler *et al*. (1963), Armstrong and Armstrong (1980) and Swanson and Van Gundy (1985). Unfortunately, these differential varieties of Hare are no longer available. Race 4 was described from California in 1999 (Smith *et al.* 1999).

Previous work by Conde and Arao-Arao (2003) showed that the three strains ('plum', 'white' and 'pink') belong to three different vegetative compatability groups (VCGs). However, it is not known how these three strains relate to the only Fot isolate that has been given the VCG number, 0060 (Katan and Di Primo 1999, Katan 1999), which is based on publications by Puhalla (1985) and Correll *et al.* (1987). Molecular methods particularly polymerase chain reaction (PCR) have been utilised to resolve genetic diversity among isolates within or between formae speciales of *Fusarium oxysporum* (McDonald, 1997). Techniques such as random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) of ribosomal DNA (rDNA) is widely used for genetic diversity studies, genome mapping and molecular diagnostics (Manulis *et al.* 1994; Annamalai *et al*. 1995). Genetic variation based upon the rDNA is commonly used for taxonomic and phylogenetic studies since sequence data for conserved and variable regions are freely available. The rDNA operons consist of conserved genes and highly variable intergenic spacer regions, the latter can be used to examine closely related taxa using RFLP (Appel and Gordon 1995; Edel *et al*. 1995).

Thus far, the use of rDNA characterisation has differentiated *F. avenaceum*, *F. arthroporioides*, *F. tricinctum* (Yli-Mattila *et al.* 2002), *F. culmorum* (Mishra *et al.* 2002) and *F. verticillioides* (Patiño *et al.* 2006). Similar studies have also been conducted for *F. oxysporum* f. sp. *dianthi* (Manicom *et al.* 1990), *F. oxysporum* f. sp. *gladioli* (Mes *et al.* 1994), *F. oxysporum* f. sp. *vasinfectum* (Fernandez *et al*. 1994), *F. oxysporum* f.sp. *cyclaminis* (Woudt *et al.* 1995), *F. oxysporum* f. sp. *melonis* (Appel and Gordon, 1995), *F. oxysporum* f. sp. *phaseoli* (Woo *et al.* 1996) and *F. oxysporum* f. sp. *fragariae* (Nagarajan *et al.* 2004). RAPD fingerprinting has previously been conducted for *F. oxysporum* f. sp. *albedinis* (Fernandez and Tantaoui 1994), *F. oxysporum* f. sp. *cubense* (Bentley *et al.* 1995), *F. oxysporum* f. sp. *erthroxyli* (Nelson *et al.* 1997), *F. oxysporum* f. sp. *lycopersici* (Mes *et al.* 1999) and *F. oxysporum* f. sp. *radicis-cucumerinum* and *F. oxysporum* f. sp. *cucumerinum* (Vakalounakis and Fragkiadakis 1999). There is only one previously published study on molecular characterization of Fot, this being the study by Troisi *et al*. (2010). These authors utilised Fot isolated from *Gerbera jamesonii*.

In the NT, three *Fot* strains with three different colours (white, pink and plum) were isolated from *Vigna unguiculata* subsp. *Sesquipedalis* with wilt symptoms. Their races and VCGs are unknown.

The importance of the disease.

Fusarium wilt (Fot) was found affecting the GPK variety of snake bean in the NT in 1999. In common with other *Fusarium* wilts, once the soil is infested it remains infested for years because of the resistant chlamydospores produced by the fungus. After slumping for several years due to the effects of the *Fusarium* disease, production increased again in 2005 with a record production of 690 tonnes worth $3 105 000 due to farmers moving to new uninfected land because of the incentive of very high market prices (Greg Owens, NT DPIFM pers. com). These new areas of land became

infected with Fot due to Fot contamination of vehicles, farm implements or farm produce, or due to the use of Fot contaminated seed. This was evidenced by the 2006 production figures of 300 tonnes, a reduction of 55 % from the 2005 figure of 690 tonnes (NT Government statistics). Similarly farms in SE Queensland may become infected due to the movement of farmers from the NT, as has happened in the past.

There are no known chemical or cultural controls for this soilborne disease. The incidence of the disease is increasing every year and virtually all producing farms are now affected. Many farms have ceased growing snake bean as losses from the disease makes production unviable. The viability of the remaining farms is being eroded as the effect of the disease spreads and intensifies. This disease is also a major threat to the Queensland snake bean industry. A race of Fot in the USA also causes major crop losses in soybeans (Nyvall 1999). In the absence of a commercially suitable resistant variety of snake bean, growers had tried unsuccessfully to manage or minimize the effects of the disease by applying farm hygiene principles, trialling fungicides such as benomyl, and moving to new land. These measures have largely proved unsuccessful. Because of high prices for snake beans in 2004, farmers moved to new land in 2005 to take advantage of the high prices. After an initial rise in production that year in 2005, there was a dramatic fall in production in 2006 because farmers had inadvertently taken the disease with them to the new land.

Research history and objective of the current study

The Northern Territory's Department of Regional Development, Primary Industry, Fisheries and Resources (NT DRDPIFR) and its predecessors has been conducting a program of surveys to identify the extent of the problem and investigate control options. Previous unpublished work established that none of the 74 snake bean lines screened for resistance had both sufficient resistance and desirable culinary qualities acceptable in the market. Several cowpea lines had good levels of resistance. Orton in 1902 reported the cowpea cultivar 'Iron' to be resistant to Fusarium wilt and demonstrated that this variety could be used as a source of resistance in breeding programs. A technique of grafting the commercial snake bean variety on to Iron cowpea was developed (Condé *et al,* 2010a). Iron cowpea has strong resistance to all the strains of *Fusarium* wilt in Darwin, and has the added advantage of resistance to root knot nematodes. The grafting technique was promoted amongst the snake bean farmers from 2004 to 2006 by Geoff Walduck and Greg Owens as part of the departmental extension strategy, however, large scale demonstration sites and training programs were required to encourage uptake of the technique.

This component of the project sought to demonstrate to snake bean growers the effectiveness of grafting on to the resistant Iron cowpea root stock as a means of controlling *Fusarium* wilt. The feasibility of adapting this method of grafting to manage soilborne diseases in other vegetable crops in Australia was also reviewed. The three Fot strains isolated from NT snake bean crops were characterised by the reaction of differential host plants and using molecular methods.

4.2 Materials and Methods

4.2.1 Grafting workshop and farm visits

A grafting workshop was held by Mark Traynor and Barry Condé at the beginning of the project (Darwin, Wednesday 24 September 2008) to demonstrate effective grafting technique and allow local growers to practice grafting and to obtain resistant Iron cowpea root stock. This was followed by visits to local snake bean growers to discuss application of the technique in their enterprise.

4.2.2 On-farm field trials

Farmers were sought out who had the snake bean *Fusarium* wilt problem and were interested in having on-farm trials of rows of grafted and seedling snake beans on their farms. Three sites were chosen for the 2009 trials. The Berry Springs site was known to have a serious *Fusarium* wilt problem. The Acacia Hills site was known to have at least moderately severe *Fusarium* wilt. The third site at Humpty Doo was chosen because it had low levels of *Fusarium* wilt. A fourth site was established at Lambells Lagoon, however, this farm had severe *Pythium* infection and so was not included as a Grafting Vs seedling trial site.

Grafted and seedling plants were grown for the on-farm trials at Berrimah Farm, Berrimah, Darwin and transported to the co-operating farms. Grafted snake beans were produced as per Conde *et al.* (2010a). Seed of the Iron cowpea rootstock was sown in pasteurized potting mix in bleach sterilized trays 1 week prior to sowing the seed of the GPK snake bean.

Below is a summary of important points to note to ensure successful grafting of snake beans:

- The growing equipment needs to be kept off the ground and away from soil splash contamination.

- When planting seeds in trays use clean potting mix – not soil
- Label trays either snake bean or cowpea to avoid mistakes at grafting time.
- Cowpea takes about 20 days to reach grafting size but the snake bean only takes about 15 days. Plant the cowpeas about a week earlier than the snake beans.
- A simple wedge graft held with a grafting clip works well.
- Graft high on the cowpea rootstock just below the first leaves.
- Make sure both cowpea and snake bean seedlings are well watered before grafting.
- Cut back the leaves from the snake bean scion to minimise moisture loss.
- Post-grafting care is important. Use wind protection and misting jets for a week or so until tips begin to shoot.
- Remove grafted plants from the mist and "harden up" for a week or so before planting in the field.

Planting dates and row sizes for the three trial sites properties are:

Site 1 - Berry Springs
Trial planted on 11/5/2009
4 alternate rows of 42 grafted and 42 seedling plants.
Totals of 84 grafted and 84 seedling plants.

Site 2 - Acacia Hills
Trial planted on 4/5/2009
4 alternate rows of 75 grafted and 75 seedling plants.
Totals of 150 grafted and 150 seedling plants.

Site 3 - Humpty Doo
Trial planted on 12/5/2009
4 alternate rows of 53 grafted and 53 seedling plants.
Totals of 106 grafted and 106 seedling plants.

Plants were assessed weekly for symptoms of disease or death due to *Fusarium* wilt from the time plants first showed symptoms of wilting. Disease was confirmed by nicking the plant towards the base to reveal the brown vascular tissue characteristic of the *Fusarium* wilt disease. Representative samples labelled with the plant number were taken back to the Plant Pathology laboratory for isolations on to PDAL (PDA medium acidified with two drops of lactic acid per 15ml Petri dish) to confirm the presence of the Fot fungus. *Fusarium* isolates obtained from samples were classified as 'pink', 'white' or 'Plum' based on the colour of the culture. Yields from the grafted and non-grafted rows were recorded in co-operation with the collaborating growers.

On-farm farmer field days were conducted to demonstrate to interested farmers the advantage of grafted plants in terms of longer plant life, reduced plant death and increased bean production. Field days were held at sites 1 and 2 in conjunction with the on-farm field trials there during 2009.

In 2010 three demonstration blocks were established. Trays of 40 grafted and seedling plants were given to growers to plant on farms at Webb Road, Duff Road and Wanderrie Road. Each of these growers had a history of Fusarium wilt on their farms. A field day was held on 25th November 2010.

4.2.3 Isolate collection and characterisation

127 samples were collected from the 2009 on-farm trial sites and other farms during 2009. These were processed in the laboratory. Isolations were made from these samples in the laboratory on to PDAL, or if *Pythium* was suspected as a cause, on to PDA. The cultures were processed as single spore cultures and stored as filter paper (Correll *et al.* 1986) cultures in the refrigerator at 4ºC. Old isolates, including the standard 'plum' (24946), 'pink' (26571) and 'white' (26536) and other old cultures stored on filter paper in the refrigerator (4º C) were used in the characterization of the strains. The majority of isolates collected since 1999 were classified as the 'pink' strain, with fewer numbers of the 'white' strain and the 'plum' strain.

4.2.4 Isolate characterisation/race determination

The screening technique used to screen the strains against the various sets of race differentials was adapted from that described by Armstrong & Armstrong (1980), and also by Haglund & Kraft (1979), Swanson & VanGundy (1985) and Woo *et al.* (1996). The method was adapted to include an agar culture inoculation technique used previously to screen snake bean and cowpea lines for resistance to the three strains of Fot (Conde *et al*. 2003). This inoculation technique was selected as it enables an accurate amount of inoculum (as number of agar plates) to be produced easily and

efficiently, and is useful when several inoculations with different culture isolates are involved.

The three standard isolates of 'plum' strain (NTP-Dc24946), 'white' strain (NTP-Dc 26536) and 'pink' strain (NTP-Dc26571) were reisolated from cultures stored as filter paper cultures (Correll *et al.* 1986) in vials in the fridge at 4ºC. Seed of the differential varieties was obtained from the Australian Tropical Crops & Forages Genetic Resource Centre, Biloela, Queensland from their seed store, or imported by them from USA. To save time, the differential lines used by Patel and Ehlers, Rigert and Harris and those drawn up from literature by Condè were all screened for resistant or susceptible reaction to the three Fot strains at the one time. Cowpea screenings for the three strains were done on three separate benches to avoid cross contamination. Other cowpea lines were also screened against the three strains in case further lines were required to differentiate between the strains. These further lines included several TVu lines previously used to investigate a cowpea virus, NT cowpea lines obtained from Arthur Cameron (Agronomist, Dept of Resources, Darwin) and other cowpeas obtained locally and from seed companies. These cowpea lines and varieties are listed in Table 4.6 (see results section 4.3.4). Due to time and seed constraints, in the majority of cases only one replicate was screened. At least two replicates (Katan *et al*. 1994) should be screened to eliminate differences due to natural variation.

The cowpea differential and potential lines were screened as composites of 15 plants in 6-inch pots. Twenty seed of each variety which was to be screened for resistance were sown as a composite in a five-inch squat pot or a four inch pot. Each screening test included a pot of GPK snake bean or Eden cowpea seedlings as the respective positive and negative control. When the seedling roots were considered strong enough, the weakest plants were culled. The fifteen strongest plants were prepared for inoculation. The root mass with potting mix was lifted off the pots and the roots trimmed with scissors. Fresh virulent cultures grown on 2 plates of PDAL were cut out and placed on potting mix at the base of six inch pots. PDAL is defined as 15ml of PDA in 9 cm plastic dishes amended with two drops of 25 % lactic acid. These acidified plates reduced bacterial contamination and also produced well defined characteristic colonies for each of the three strains. The composite seedlings with trimmed roots were placed on the cultures and the plants potted up with a commercial potting mix obtained from interstate to avoid the risk of Fot contamination from any local potting mix. Inoculated plants were taken to another shade house, Osmocote® slow release fertilizer added and the plants were watered. Negative uninoculated control pots of Eden cowpeas (a very susceptible plant) and other lines were maintained in another shadehouse and a hot glasshouse to avoid contamination issues.

Symptoms usually began to appear in the GPK or Eden positive control plants about four weeks after inoculation. Results were read on the test cowpea seedlings approximately eight weeks after inoculation, as external wilt symptoms and internal symptoms of vascular discoloration. The reaction of the differentials to the specific isolates of Fot was classified as resistant (no observable disease) or susceptible (dead plant) (as per Armstrong and Armstrong 1980; Haglund and Kraft, 1979). Resistance and susceptibility was confirmed by observation of internal symptoms and isolation of the pathogen from affected plants 7 cm from the soil level. Isolations from the plants were compared with each other and cultures of the standard isolates. Results were recorded as fractions of number of wilted plants / number of plants tested as per Armstrong and Armstrong (1980).

4.2.5 Molecular characterisation

DNA extractions

A total of 25 Northern Territory Fot isolates listed in Table 4.1 were used for molecular characterisation. DNA was extracted from single spore lawns grown on duplicate 90 mm PDA plates. These were compared with Fot Races 1, 2, 3 and 4. Cultures of Races 1 and 2 were obtained from Cryosite Distribution (Australia). DNA from Races 3 and 4 was imported from the University of California, Riverside. The *Fusarium* DNA was extracted using the DNeasy plant mini kit (Qiagen, Australia) following the manufacturer's instructions.

Table 4.1. *Fusarium oxysporum* f. sp. *tracheiphilum* isolates used in the genetic diversity studies.

Sample number	Isolate culture number	Location (Source)	Colour
NTP-Ds11331	NTP-Dc26536 (standard)	Lambells Lagoon Rd, NT	White
NTP-Ds19607	NTP-Dc34326	Walter Rd, Acacia, NT	White
NTP-Ds19588	NTP-Dc34334	Buckley Rd Site 5, Humpty Doo, NT	White
NTP-Ds19658	NTP-Dc34391	Connelly Rd Site 1, Lambells Lagoon, NT	White
NTP-Ds13316	NTP-Dc34941	Barr Rd, Marrakai, NT	White
NTP-Ds11333	NTP-Dc34963	Ewart Rd, Lambells Lagoon, NT	White
NTP-Ds19926	NTP-Dc34970	Connelly Rd Site 2, Lambells Lagoon, NT	White
NTP-Ds11346	NTP-Dc26571 (standard)	Arnhem Hwy, NT	Pink
NTP-Ds19456	NTP-Dc34308	Monck Rd Site 2, Acacia, NT	Pink
NTP-Ds19599	NTP-Dc34322	Mulgara Rd, Berry Springs, NT	Pink
NTP-Ds19606	NTP-Dc34325	Walter Rd, Acacia, NT	Pink
NTP-Ds19712	NTP-Dc34457	Buckley Rd Site 5, Humpty Doo, NT	Pink
NTP-Ds19728	NTP-Dc34506	Wanderrie Rd, NT	Pink
NTP-Ds19762	NTP-Dc34517	Monck Rd Site 1, Acacia, NT	Pink
NTP-Ds12275	NTP-Dc34946	Shady Ct, Leayner, NT	Pink
NTP-Ds13317	NTP-Dc34950	Barr Rd, Marrakai, NT	Pink
NTP-Ds12341	NTP-Dc34965	Old Bynoe Rd, Darwin River, NT	Pink
NTP-Ds19925	NTP-Dc34969	Connelly Rd Site 2, Lambells Lagoon, NT	Pink
NTP-Ds10007	NTP-Dc24946 (standard)	Walter Rd, Acacia, NT	Plum
NTP-Ds10246	NTP-Dc24947	Buckley Rd Site 1, Humpty Doo, NT	Plum
NTP-Ds10339	NTP-Dc24948	Buckley Rd Site 4, Humpty Doo, NT	Plum
NTP-Ds10385	NTP-Dc24949	Buckley Rd Site 2, Humpty Doo, NT	Plum
NTP-Ds10245	NTP-Dc24950	Buckley Rd Site 3, Humpty Doo, NT	Plum
NTP-Ds19652	NTP-Dc34383	Walter Rd, Acacia, NT	Plum
NTP-Ds15095	NTP-Dc34952	Jefferis Rd, NT	Plum
	Fot race 1 ATCC+ 16608		
	Fot race 2 ATCC+ 16609		
	Fot race 3*		
	Fot race 4*		

+ Cultures obtained from Cryosite Distribution (Australia).
* DNA provided by Dr. Philip Roberts (University of California, Riverside).

Intergenic spacer (IGS) region PCR

The intergenic spacer region (IGS) was amplified using the PCR primers CNL12 (5' CTG AAC GCC TCT AAG TCA G 3') and CNS1 (5' GAG ACA AGC ATA TGA CTA CTG 3') (Geneworks, Australia; White *et al.* 1990, Anderson and Stasovski 1992). PCR reactions were conducted in a 50 µL mixture containing 1 X ImmoMix Red with 3 mM $MgCl_2$ (Bioline, Australia) and 0.2 µM of each primer and 5-10 ng of DNA template. The PCR conditions used were initial enzyme activation of 95 °C for 10 min; followed by 35 cycles of denaturing at 95 °C for 1 min, annealing at 51 °C for 1 min, extension at 72 °C for 1.5 min and final extension of 72 °C for 10 min. The 2.6 Kb PCR product was separated on a 3 % agarose/ethidium bromide gel using 1 X TAE buffer and visualised under UV light.

Restriction fragment length polymorphism

In separate 10 µL reactions, approximately 1 µg of 2.6 Kb IGS PCR product was digested with I U of the following enzymes; *Cfo* I (Promega, Australia), *Ava* II, *Eco* RI and *Sau* 3A (New England Biolabs, Australia) according to the manufacturer's instructions. All digestions were conducted at 37 °C for either one hr or overnight. The digested fragments separated in 8 % polyacrylamide for 5 hr at 150 V. The gel was stained in 10 mg/mL ethidium bromide bath for five min, and destained in distilled water for ten minutes and visualised under UV light.

Random Amplified Polymorphic DNA

Twenty ten-base oligonucleotide primers from Operon primer kit A as described by Hyun and Clark (1998) and Nagarajan *et al.* 2004 were used to determine polymorphism within and between the isolates (Table 4.2).

Table 4.2. Oligonucleotide primers used for RAPD genetic diversity analysis.

Oligonucleotide primer name	Sequence (5' to 3')
OPA-1*	CAG GCC CTT C
OPA-2*	TGC CGA GCT G
OPA-3*	AGT CAG CCA C
OPA-4	AAT CGG GCT G
OPA-5*	AGG GGT CTT G
OPA-6	GGT CCC TGA C
OPA-7*	GAA ACG GGT G
OPA-8*	GTG ACG TAG G
OPA-9*	GGG TAA CGC C
OPA-10	GTG ATC GCA G
OPA-11	CAA TCG CCG T
OPA-12	TCG GCG ATA G
OPA-13	CAG CAC CCA C
OPA-14	TCT GTG CTG G
OPA-15*	TTC CGA ACC C
OPA-16	AGC CAG CGA A
OPA-17	GAC CGC TTG T
OPA-18*	AGG TGA CCG T
OPA-19	CAA ACG TCG G
OPA-20*	GTT GCG ATC C

*RAPD primers used for further diversity studies.

PCR reactions were conducted in a 25 µL mixture containing 1 X ImmoMix Red with 3 mM $MgCl_2$ (Bioline, Australia) and 0.2 µM of primer and 5-10 ng of DNA template. PCR conditions used were initial enzyme activation of 95 °C for 10 min; followed by 40 cycles of denaturing at 94 °C for 30 s, annealing at 40 °C for 1 min, extension at 72 °C for 2 min and final extension of 72 °C for 10 min. The PCR product was separated on a 1.5 % agarose/ethidium bromide gel using 1 X TAE buffer for 2 h at 80V. The gel was then stained in 10 mg/mL of ethidium bromide and destained in distilled water before visualisation under UV light.

For RAPD data analysis, each profile was compared on the basis of the presence (1) versus the absence (0) of an amplified band. Using the web-based program, DendroUPGMA (http://genomes.urv.cat/UPGMA), a genetic similarity matrix was calculated. Similarity coefficients were transformed into distances to produce a clustering using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm. The Jaccard (Tanimoto) coefficient was used to compare between the set of variables, the phylogenetic dendrogram was based upon bootstrap replications of 100 times. The cophenetic correlation coefficient which measures how faithfully a dendrogram preserves the pairwise distance between the data points was calculated.

Multigene characterisation - EF1-α, ß tubulin and histone 3 PCR

Fusarium spp. can be characterised by using multigene sequences, particularly, the translation elongation factor gene 1α (TEF1α); β tubulin and the histone 3 genes. Details of the primer sequences and PCR conditions used are summarised in Table 4.3.

Table 4.3. PCR primers for *Fusarium* spp. multigene characterisation and their associated cycling conditions.

Gene	Primer combination (5'- 3')	PCR cycling conditions
TEF1α+	EF1 - (ATGGGTAAGGA(A/G)GAC AAGAC) EF2 - (GGA(G/A)GTACCAGT(G/C)ATCATGTT)	Step 1. 95 °C/10 min Step 2. 94 °C for 1 min Step 3. 55 °C for 1 min Step 4. 72 °C for 2 min Step 5. Repeat Steps 2- 4, 40 times.
Histone 3#	H3-1a (ACTAAGCAGACCGCCCGCAGG) H3-1b (GCGGGCGAGCTGGATGTCCTT)	Step 1. 95 °C/10 min Step 2. 92 °C for 1 min Step 3. 68 °C for 1 min Step 4. 72 °C for 1 min Step 5. Repeat Steps 2- 4, 30 times. Step 6. 72 °C for 5 min
β tubulin*	T1 (AACATGCGTGAGATTGTAAGT) T2 (TAGTGACCCTTGGCCCAGTTG)	Step 1. 95 °C/10 min Step 2. 94 °C for 1 min Step 3. 53 °C for 1 min Step 4. 72 °C for 1 min Step 5. Repeat Steps 2- 4, 35 times. Step 6. 72 °C for 5 min

+ O'Donnell *et al.* (1998)

Glass and Donaldson (1995)

* O'Donnell and Cigelnik (1997)

Multigene characterisation - Sequencing

The PCR products were purified using the QIAquick PCR purification kit (Qiagen, Australia) according to the manufacturer's instructions. The purified PCR products were eluted in 30 µL sterile distilled water pH 7.3. DNA concentration was determined using agarose gel electrophoresis with the low mass ladder (Invitrogen, Australia) as a reference. The products were sequenced using the Big Dye Terminator Mix (Bioscience North Australia, Darwin, NT). Nucleotide sequences were analysed and edited using Geneious Pro 5.0.3. Sequences were deposited into the GenBank database and sequence similarities were conducted using the BLASTN database (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). Sequence data will be deposited into GenBank.

4.3 Results

4.3.1 Grafting workshop and farm visits

Seven farmers attended and took part in the grafting workshop coducted by Mark Traynor and Barry Condé as part of the soilborne diseases workshop held by the project team in Darwin on Wednesday 24 September 2008. Four of these agreed to participate in the on-farm demonstration program. A further five vegetable farms were visited by the team the following day. Strong interest in the workshop resulted in the production of a DVD explaining the need for grafting and demonstrating the grafting technique.

4.3.2 On-farm field trials and demonstrations

Plants on the 2009 trial sites 1-3 grew well with few problems except for the *Fusarium* wilt, Cercospora leaf spot, caused by *Pseudocercospora cruenta* (Sacc.) Deighton and bean fly (*Ophiomyia phaseoli*). Bean fly required control measures from seedling emergence to prevent plants being lost due to this pest.

Infection rates

The grafted plants remained uninfected at all 3 sites for the duration of the trials. Ungrafted seedlings showed different rates of infection and death over time at the three sites due to different levels of *Fusarium* present in the soils (Fig. 4.1). The seedlings at site 1 showed signs of infection as early as 30 days after planting (DAP). Infection and death increased rapidly until all seedlings were dead by 78 DAP. The seedlings at this site became infected and died before any harvest. Seedling infection at site 2 commenced early (44 DAP) but was slower to develop compared to site 1 (Fig. 4.1). No infected seedlings were recorded at site 3 until 71 DAP and the rate of infection was slow compared to the other sites.

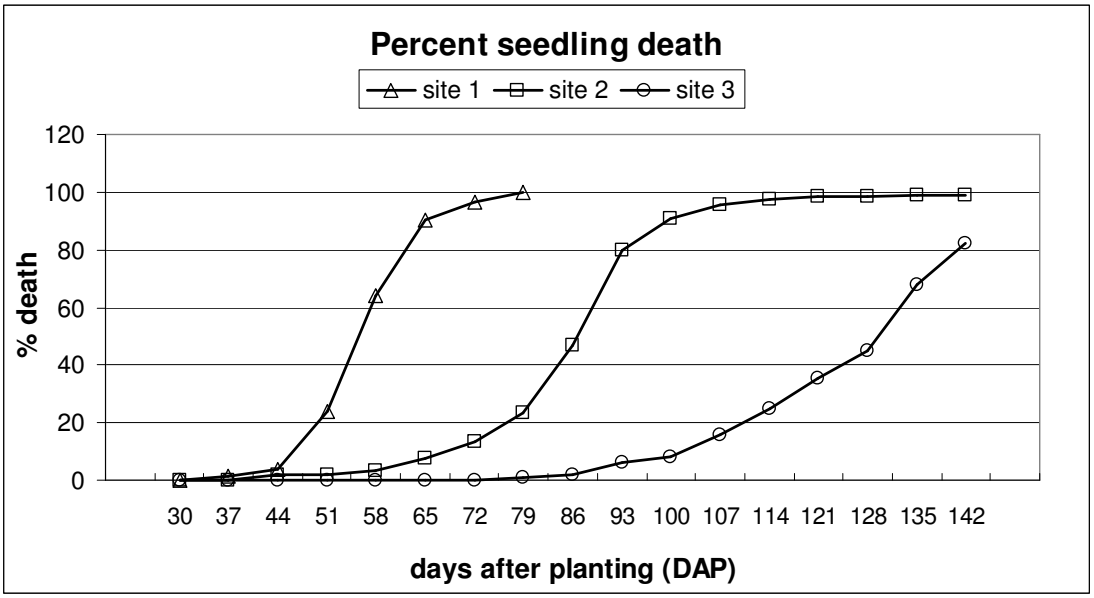


Figure 4.1. Percent seedling deaths (ungrafted seedlings) over time for sites 1, 2 & 3..

Yields

The yield data shows that the benefit of grafted plants was dependant on how quickly the seedling plants became infected and stopped production. The collaborating growers recorded the row yields for the ungrafted seedlings and grafted plants at sites 2 and 3. The ungrafted seedlings at site 1 produced no commercial harvest and the grower did not record yield from the grafted plants at this site. The cumulative yield data for site 2 shows higher yield from grafted plants from the start of harvest (Fig. 4.2). At 80 DAP, seedling death increased rapidly with 80 % of seedlings dead by 90 DAP (Fig. 4.1 & 4.4). Yield from the seedling rows stopped after 85 DAP while the grafted plants continued to yield well until 105 DAP (Fig. 4.2). This was also evident at site 3 where seedling yield dropped away as seedling death rate increased (Fig. 4.3 & 4.1).

These trials indicate that where infection is moderate to severe (site 2) grafted plants produce more than twice as much as ungrafted seedlings (Table 4.4). Where infection is low (site 3) the increased yield per plant resulting from grafting, approximately 15 % (Table 4.4) is unlikely to be sufficient to offset the extra labour costs involved in producing the grafted plants.

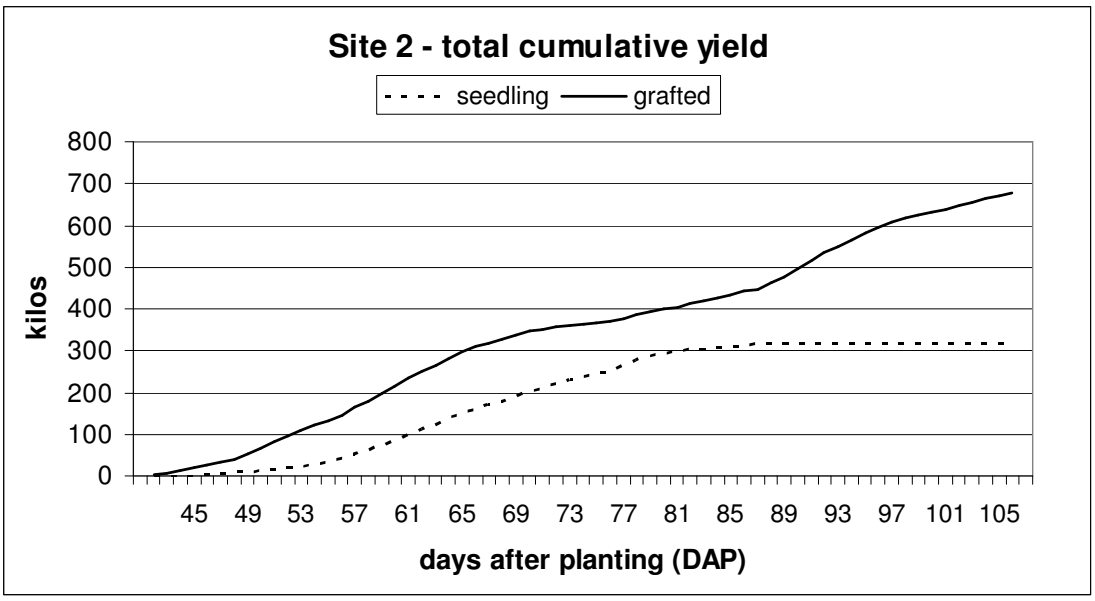


Figure 4.2. Total cumulative yield of ungrafted seedling and grafted plants over time (days after planting) for Site 2 (moderate-severe *Fusarium* wilt infection in non-grafted seedlings).

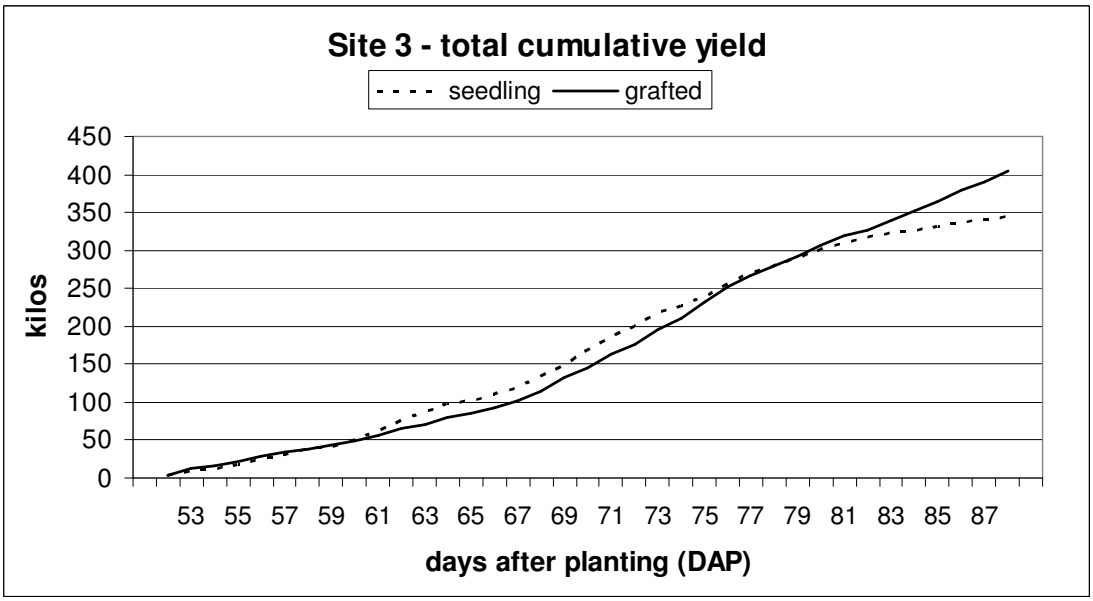


Figure 4.3. Total cumulative yield of ungrafted seedling and grafted plants over time (days after planting) for Site 3 (low Fusarium wilt infection in non-grafted seedlings).

Table 4.4. Average seedling yield per plant, average grafted yield per plant, and percent increase in yield for grafting for Site 2 (moderate-severe infection in non-grafted seedlings) and for Site 3 (low infection in non-grafted seedlings).

	Seedling yield / plant	Grafted yield / plant	% increase in yield
Site 2	2.1 kg	4.5 kg	114 %
Site 3	3.3 kg	3.8 kg	15 %

Figure 4.4. Symptoms of Fusarium wilt caused by the fungus, *Fusarium oxysporum* f.sp. *tracheiphilum* on non-grafted snake beans. Brown discoloration of the vascular tissue near the base of a plant, a characteristic symptom of disease (left) and early dying seen here as alternating rows of dead non-grafted plants and healthy grafted plants at trial site 2 (right).

A fourth trial site was abandoned due to high levels of *Pythium* base rot caused by the fungus, *Pythium myriotylum*, Drechsler. Identification to species was done by Len Tesoriero and Leanne Forsyth, Industry and Investment NSW, Elizabeth MacArthur Institute. The incidence of the *Pythium* base rot in twelve rows is shown in Table 4.5. Previously *Pythium* base rot has been a problem with snake beans only where they were waterlogged for some reason, such as excessive irrigation, poor drainage and inadequate soil ripping preparation prior to planting.

Table 4.5. Counts of plants, *Pythium* base rot affected plants and percentage *Pythium* base rot in 12 rows at Site 4.

Row No	No of Plants	Pythium base rot	Percent Base rot
1	118	30	25.4
2	120	27	22.5
3	119	32	26.9
4	119	22	18.5
5	119	26	21.8
6	121	33	27.3
7	122	40	32.8
8	122	24	19.7
9	120	24	20.0
10	121	23	19.0
11	119	23	19.3
12	121	26	21.5
Totals	1441	330	22.9

Two field days were held in conjunction with the on-farm field trials, Nine people including three farmers attended the first field day at Site 1 on 15 July 2009. Eight people including five farmers attended the second field day at Site 2 on 5 August 2009.

Growers who planted demonstration blocks during 2010 reported that grafted plants remained healthy and produced beans whilst the seedling plants died. The field event on 25th November was attended by 50 people. Topics covered included grafting and post-graft care, including rapid, cost-effective establishment of a misting tent required for post-graft seedling maintenance. Additional sessions included use of green manures, organic matter, soil health, root knot nematode management, tillage and hardpans, use of registered chemicals, cucurbit mosaic viruses and insect management in vegetable crops. Attendees were provided with a pack of information on the topics covered, including a DVD on grafting snake bean and Agnotes on growing green manure crops, root knot nematode, virus diseases of cucurbits and soil and root problems of snake bean.

The ABC Country Hour attended the event, broadcasting interviews from participants in the midday program of the 25th November.

4.3.3 Isolate cultures

The ‘white’ and ‘pink’ strains whilst different in colour were similar having abundant fluffy (Puhalla 1985) cultures on PDAL. In contrast, the ‘plum’ strain has flat colonies on PDAL, having a distinct inner and outer zone structure. ‘White’ strains are always white, but may age to a pale beige colour with a small black area in the centre or with black zones to the culture. Typically ‘plum’ cultures begin as small plum coloured colonies and age to a purple colour. There is however variation in colour in the ‘plum’ and ‘pink’ isolates, even in an individual isolate after one passage through plants. It is suggested that the genetics of the plant may affect the colour of the colonies. Occasionally ‘plum’ and ‘pink’ isolates may appear similar in colour, but are differenced by their differing colony structure. ‘Plum’ colonies are always flat on PDAL, whilst ‘pink’ colonies are always fluffy with abundant aerial mycelium.

4.3.4 Race determination

Tables 4.6, 4.7, 4.8 and 4.9 record the reactions of the three sets of cowpea race differentials and a set of potential race differentials to the three strains of Fot in Darwin, the ‘plum’ strain, the ‘white’ strain and the ‘pink strain’, represented by the standard isolates, NTP-Dc24946 (‘plum’ strain), NTP-Dc 26536 (‘white’ strain) and NTP-Dc26571 (‘pink’ strain). Wilt disease symptoms consisted of cessation of growth, flaccid upper leaves and a bright yellow veinal chlorosis on the lower leaves. Internally a reddish-brown vascular discolouration, often appearing as a ‘dirty stem’ was evident. Sometimes this symptom was evident only on one-side of the plant. Some entries in Tables 4.6 and 4.7 are supplemented with 2002 and 2003 results to provide a complete data set.

Table 4.6 shows the reactions of the ‘PN Patel and Jeff Ehlers’ set of Race Differential cowpea lines to inoculation with the standard isolates of the three Fot strains from Darwin, compared with the known reactions of these cowpea race differentials to Races 1, 2, 3 and 4 in California, USA. The ‘PN Patel and Jeff Ehlers’ set of Race Differentials is the most comprehensive set. It includes differential reactions to all four known races and consequently is considered the most appropriate set to investigate initial similarities and differences to races 1 to 4.

The reactions of the 'plum' strain (NTP-Dc24946) on the Ehlers- Patel Race Differentials (Table 4.6) are consistent with those of Race 2. The reactions of this strain on the Condè set of differentials (Table 4.7) is also generally consistent with Race 2. The 'plum' strain caused some infection in CB 27. Unfortunately the reaction of CB 27 to races 1 and 2 is unknown (Jeff Ehlers, Marti Pottorff, pers. com.). Molecular characterisation studies (4.3.5) suggest that the 'plum' strain is similar to, but not identical with Race 2. This is supported by the susceptible reaction of Mississippi Silver, a variety, known to be resistant to Race 2, following inoculation with the 'plum' strain. The relationship of the 'plum' strain to Race 2 can only be further resolved by inoculating the 'plum' strain on to a series of cowpeas with known reactions to Race 2, in order to identify cowpeas with any differences between the 'plum' and Race 2 reactions. Armstrong & Armstrong 1950 and Marti Pottorff (pers. com.) have available lists of cowpeas with known reactions to Race 2.

Both the 'white' (NTP-Dc26536) and 'pink' (NTP-Dc26571) strains were able to kill the differential line PI115681, which is resistant to all four known races of Fot in USA. This suggests that the two Northern Territory strains ('white' and 'pink') are two new races, provisionally numbered 5 and 6.

Table 4.6. Reactions of the 3 Darwin Fot strains ('plum', 'white' and 'pink') compared with reactions of Races 1, 2, 3 and 4 on PN Patel and Jeff Ehlers' set of Race Differentials. R is resistant, S is susceptible, all other entries refer to number of wilted plants / number of plants tested.

Cowpea line	Race 1	Race 2	Race 3	Race 4	Isolate NTP-Dc 24946 [a] ('plum')	Isolate NTP-Dc 26536 ('white')	Isolate NTP-Dc 26571 ('pink')
Groit (JE) **AusTRCF 317988**	S	R	R	R	0/15	0/15	0/15
8517 **AusTRCF 317985**	R	R	R	R	0/15	0/15	0/15
PI115681 **AusTRCF 317986**	R	R	R	R	0/15	13/15, 3/15	2/15, 4/15
Prima (JE) **AusTRCF 317989**	R	S	S	S	11/15	15/15, 15/15	10/15, 6/15
524B **AusTRCF 317984**	R	S	R	S	12/15	10/10	13/15
PI162952 **AusTRCF 317987**	S	R	S	S	0/15	8/15,4/15	7/15,9/15
GPK **AusTRCF 313545** positive control					8/13	15/15, 15/15	10/15, 9/15

[a] = 2002 results

Table 4.7 shows the reactions of the three Northern Territory strains on 'Condè's set of Differentials', compared with the known reactions of Races 1, 2, 3 and 4. Mississippi Silver was added as another differential (due to its known reactions), being resistant to Races 1, 2 and 3 (Hare 1967; PN Patel and Ehlers pers. com). The fact that the 'plum' strain infected Mississippi Silver which is known to be resistant to Race 2, provides further evidence that the Northern Territory 'plum' strain may not be Race 2.

Table 4.7. Reactions of Races 1, 2, 3 and 4 on Condé's (Condè and Arao-Arao 2003) set of Race Differentials. R is resistant, S is susceptible, all other entries refer to number of wilted plants / number of plants tested.

Cowpea line	Race 1	Race 2	Race 3	Race 4	Isolate NTP-Dc 24946 ('plum')	Isolate NTP-Dc 26536 ('white')	Isolate NTP-Dc 26571 ('pink')
CB5[1] **AusTRCF 85564**	R	S	S	S	12/15 [a]	13/13[b]	15/15 [b]
TVu 347[2] **AusTRCF 84805**	R	R	S	-	0/15 [a]	0/15	1/15
CB46[3] **AusTRCF 312391**	R	R	R	S	0/15 [a]	0/15, 0/15	0/15, 0/15
Miss. Cream[4] **AusTRCF 321320**	R	R	R	-	0/15**,** 0/15	1/15, 0/15	0/15, 0/15
CB27[5] **AusTRCF 312392**	-	-	R	R	5/15**,** 2/15	7/15, 10/15	0/15, 0/15
Miss. Silver[6] **AusTRCF 321321**	R	R	R	-	5/15, 3/15	2/15, 0/15 (9/12 [b])	0/15, 0/15 (0/15 [b])
GPK **AusTRCF 313545** positive control					8/13 [a]	15/15, 15/15	10/15, 9/15

[a] = 2002 results, [b] = 2003 results
[1] Rigert & Forster (1987); Harris & Ferris (1991); Ehlers *et al.* (2000).
[2] Armstrong & Armstrong (1980)
[3] Smith *et al.* (1999); Ehlers *et al.* (2000)
[4] MAFES (1980)
[5] Ehlers *et al.* (2000)
[6] Hare (1967)

Table 4.8 shows the reactions of 'plum', 'white' and 'pink' strains to the 'Rigert Harris' set of Race Differentials. A shortcoming of this set of differentials is that they were compiled and used when only three races were known. Jeff Ehlers (pers com) supplied the reaction of PI162925 to race 4 as Susceptible. The reaction of PI 115683 to race 4 is unknown. The fact that the 'plum' strain infected some plants of PI162925 which is resistant to race 2, is further evidence that this 'plum' strain is different to race 2.

Reactions of the 'Rigert Harris' differentials (Table 4.8) suggest that NTP-Dc 26536 ('white') and 26571 ('pink') are race 1. However, susceptible reactions of CB 5 (which is resistant to Race 1, Table 4.7) indicate that this is not the case.

Table 4.8. Reactions of Race 1, 2, 3 on cowpea differentials used by Rigert (1985) and later by Harris (1989). R is resistant, S is susceptible, all other entries refer to number of wilted plants / number of plants tested.

Cowpea line	Race 1	Race 2	Race 3	Race 4	Isolate NTP-Dc 24946 ('plum')	Isolate NTP-Dc 26536 ('white')	Isolate NTP-Dc 26571 ('pink')
PI115683 **AusTRCF 45562**	S	R	R	-	1/15, 0/15	3/15, 4/15	3/15, 1/15
PI162925 **AusTRCF 321135**	S	R	S	S^	3/15, 4/15	7/15, 3/15	8/15, 4/15
GPK **AusTRCF 313545** positive control						15/15, 15/15	10/15, 9/15
Eden **AusTRCF 323975** positive control					15/15		

S^ - information that PI162925 is Susceptible to Race 4 from Jeff Ehlers, pers. com. November 2010.

The Northern Territory 'white' (NTP-Dc 26536) and 'pink' (NTP-Dc26571) strains could not be separated using the two differential sets supplied from the USA (Tables 4.6 and 4.8). A large number of TVu, NT accessions and other cowpea varieties (Table 4.9) were screened in an effort to separate reactions of the 'white' and 'pink' strains. Lines which showed promise in their ability to differentiate between 'white' and 'pink' are:

TVu 2740 (15/15 infected by the 'white' strain; 0/15 infected by the 'pink' strain)
TVu 3433 (14/15 infected by the 'white' strain; 0/15 infected by the 'pink' strain)
NT 2238 (15/15 infected by the 'white' strain; 0/15 infected by the 'pink' strain)
NT 2190 (15/15 infected by the 'white' strain; 0/15 infected by the 'pink' strain)
NT 5472 (15/15 infected by the 'white' strain; 0/15 infected by the 'pink' strain)

The reactions on Mississippi Silver in Condè's (Condè and Arao-Arao 2003) Differential set, Table 4.7 may also enable separation of the 'white' from the 'pink' strains.

Table 4.9. Reactions of other cowpea lines screened for their ability to differentiate between 'white' and 'pink' strains. Table entries refer to number of wilted plants / number of plants tested, highlighted lines show clear differential reactions.

Cowpea line	Isolate NTP-Dc 24946 ('plum')	Isolate NTP-Dc 26536 ('white')	Isolate NTP-Dc 26571 ('pink')
Eden **AusTRCF 323975**	15/15,15/15 [a]	13/15, 15/15	15/15, 14/15
Bora Bean **AusTRCF 324105**	0/15	1/15	0/15
Red Caloona **AusTRCF 306442**	10/15	15/15	11/15
Giant Cow **AusTRCF 324104**	0/15	0/15	0/15
Ebony Cow **AusTRCF 306570**	0/15	0/15	0/15
Meringa **AusTRCF 306446**	0/15	0/15	0/15
TVu 401 **AusTRCF 84664**	13/15	15/15	15/15
TVu 401 **AusTRCF 84809**	0/15	0/15	0/15
TVu 2657 **AusTRCF 84661**	0/15	9/15	0/15
Tvu 2740 **AusTRCF 84645**	3/15	15/15	0/15
Tvu 2845 **AusTRCF 84662**	5/15	S15/15	15/15
Tvu 3273 **AusTRCF 84870**	8/15	S15/15	4/15
Tvu 3433 **AusTRCF 84653**	S11/15	15/15	0/15
Black Seeded Thai	1/15	6/15	0/15
Croudu Butter Pea	0/15	1/15	0/15
Purple Hull Pea	0/15	8/15	0/15
NT 2103 **AusTRCF 24219**	0/10	5/15	2/10
NT 2189 **AusTRCF 324106**	0/15	6/15	2/15
NT 2190 **AusTRCF 34821**	2/15	15/15	0/15
NT 2200 **AusTRCF 39137**	13/15	15/15	3/15
NT 2223 **AusTRCF 45579**	2/15	0/15	0/15
NT 2228 **AusTRCF 45584**	1/6	8/11	4/6
NT 2233 **AusTRCF 45589**	2/15	0/15	0/15
NT 2238 **AusTRCF 50849**	13/15	15/15	0/15
NT 2436 **AusTRCF 61274**	0/15	0/6	0/15
NT 3289 **AusTRCF 306460**	0/15	2/11	0/15
NT 5471 **AusTRCF 324107**	0/5	3/11	0/4
NT 5472 **AusTRCF 80208**	0/7	15/15	0/15
NT 5473	0/15	0/15	0/15

AusTRCF 80206			
NT 5474 **AusTRCF 80215**	2/15	5/9	0/9
NT 5475 **AusTRCF 80209**	5/7	0/11	0/7
NT 6584 **AusTRCF 80199**	11/15	10/15	1/15
NT 6610 **AusTRCF 80225**	3/9	8/10	1/14
Eden III **AusTRCF 323975**		14/15	
Ein El Gazal **AusTRCF 321137**	13/15	12/15	3/15
Burkino Faso	6/8	5/8	0/8
	Isolate 24946	Isolate 26536	Isolate 26571

[a] = number of wilted plants / number of plants tested

The uninoculated pots of Eden cowpea and other uninoculated lines of cowpeas remained uninfected by Fot during the series of race inoculation experiments.

Race reaction results showed that the 'plum' strain is similar to Race 2. However, results using Mississippi Silver (Table 4.7) suggest that the 'plum' strain may be slightly different from Race 2. This needs to be confirmed by inoculating sufficient numbers of cowpeas with known reaction to Race 2. The race differential work showed that the 'white' and 'pink' strains are different from the known races 1 to 4 and are similar to each other. This result is also reflected in differences in the cultures of the three strains (see 4.3.3). The only races described outside USA are Race 1 from Nigeria (Armstrong & Armstrong 1980) and Race 3 from Taiwan (Professor Yi-Sheng Lin, pers. com 1999).

4.3.5 Molecular characterisation

DNA extractions

Fusarium DNA was extracted from all isolates as listed in Table 4.1. *Fot* races 3 and 4 were purified and supplied by University of California, Riverside. All DNA samples contained no PCR inhibitors.

Intergenic spacer (IGS) region PCR

The 2.6 Kb IGS region was amplified from all isolates from Table 4.1, except for *Fot* race 3 which consistently failed to amplify using the CNL12 and CNS1 primers. This was not a result of PCR inhibitors since amplification was successful using other primers (data not shown).

Restriction fragment length polymorphism

The DNA fingerprint using *Ava* I did not show polymorphism between the 'white', 'pink' and 'plum' standard isolates. *Fot* races 1 and 2 were indistinguishable from each other, while there was a slight polymorphism observed in *Fot* race 4 where the band shift from 1.1 Kb to 1 Kb (Fig. 4.5). *Cfo* I DNA fingerprinting indicated that the 'white' and 'pink' strains had an indistinguishable pattern, *Fot* races 2 and 4 were identical. While the 'white' and 'pink' standards were indistinguishable from *Fot* races 1 and 4 using *Eco* RI, the 'plum' and *Fot* race 2 had distinct patterns. *Sau* 3A did not give a clear DNA fingerprint banding for all isolates used in the study (Fig. 4.5).

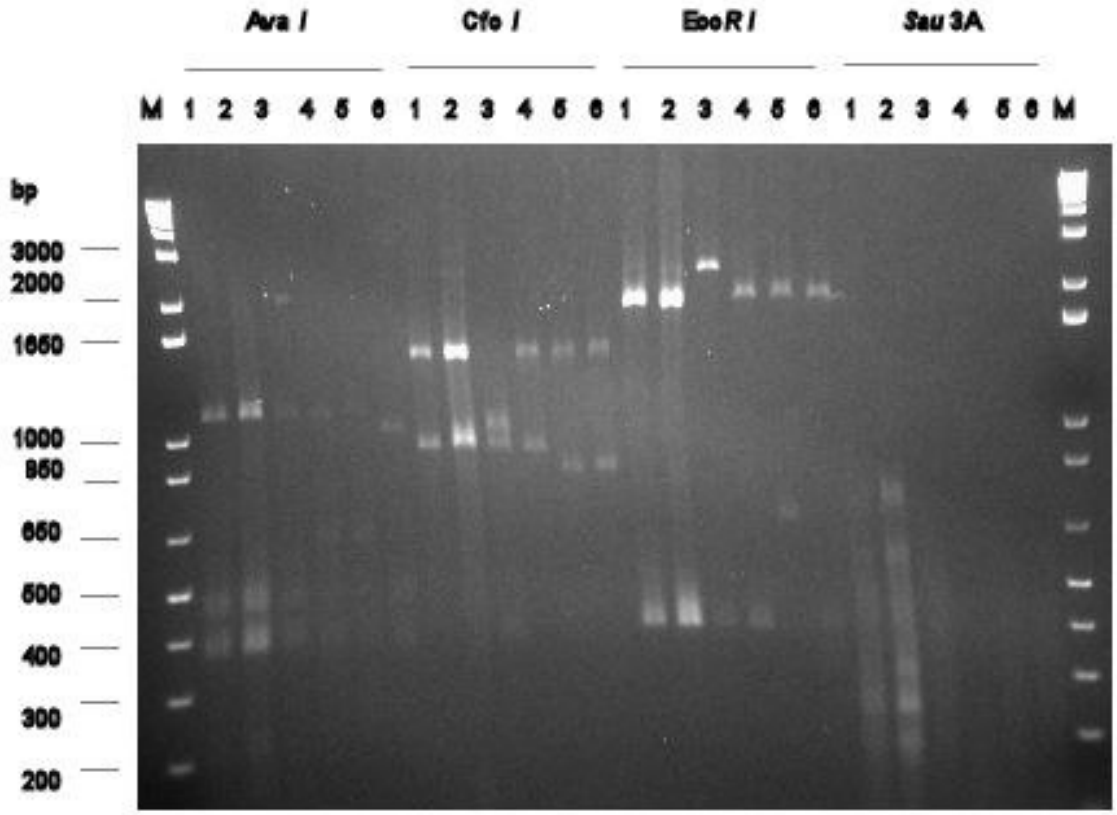
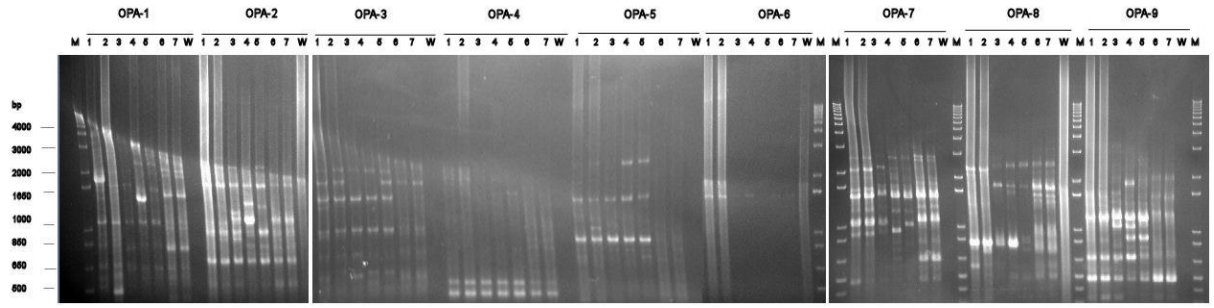


Figure 4.5. Restriction fragment length polymorphism using the following enzymes; *Ava* I, *Cfo* I, *Eco* RI and *Sau* 3A. In each set, M = 1 Kb Plus DNA marker (Invitrogen, Australia), 1 = *Fot* 'white' standard (NTP-Dc26536), 2 = *Fot* 'pink' standard (NTP-Dc26571), 3 = *Fot* 'plum' (NTP-Dc24946), 4 = *Fot* Race 1, 5 = *Fot* Race 2 and 6 = *Fot* Race 4.

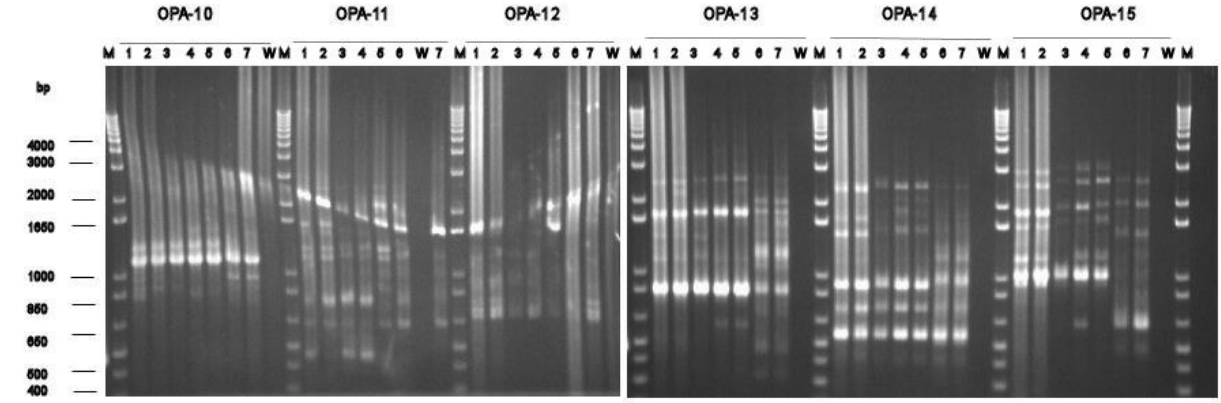
4.4. Random amplified polymorphic DNA

The 20 oligonucleotide primer used indicated that *Fot* races 3 and 4 were indistinguishable from each other with similar RAPD patterns. OPA-6 was discontinued since no clear pattern was obtained for the amplification. Of the 20 primers used, only ten showed some variations between isolates and were used to investigate the genetic diversity of *Fot* isolates, particularly those classified in the three colour strains ('white', 'pink' and 'plum') (Fig. 4.6).

(a)



(b)



(c)

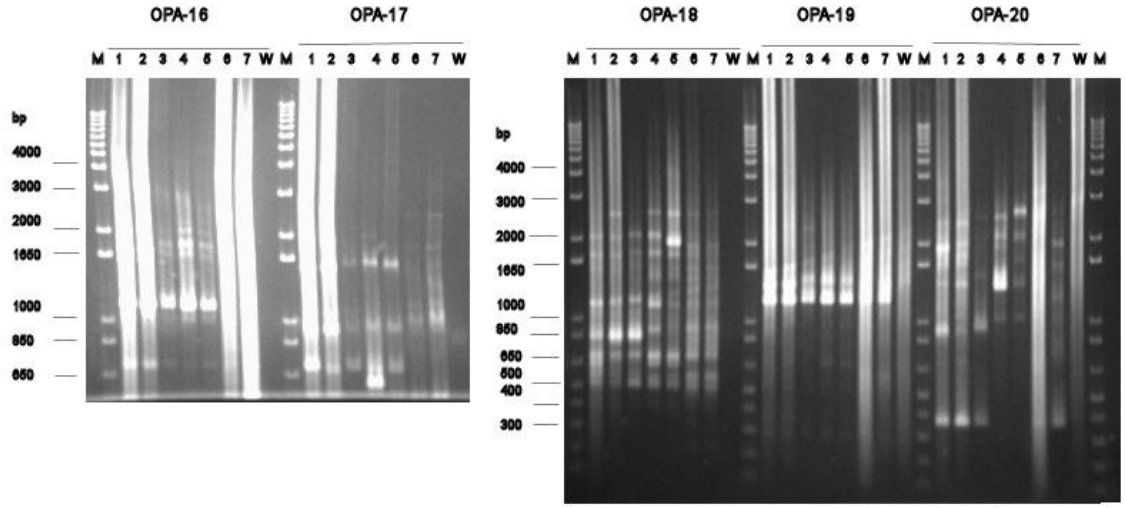


Figure 4.6 (a,b,c). Random amplified polymorphic DNA analysis using 20 different oligonucleotides. In each set, M = 1 Kb Plus DNA marker (Invitrogen, Australia), 1 = *Fot* 'white' standard (NTP-Dc26536), 2 = *Fot* 'pink' standard (NTP-Dc26571), 3 = *Fot* 'plum' (NTP-Dc24946), 4 = *Fot* Race 1, 5 = *Fot* Race 2, 6 = *Fot* Race 3, 7 = *Fot* Race 4 and W = sterile distilled water.

In all 20 RAPD patterns, the 'white', 'pink' and 'plum' standard isolates showed polymorphisms when compared with any of the references *Fot* races 1 – 4. Although some similarities were observed with *Fot* race 1 and 2, it was not consistent across patterns obtained for the 20 RAPD primers. This suggested that the three NT strains could not be classified as *Fot* races 1 – 4 but are potentially new races.

A maximum of 12 bands were observed and a minimum of five were polymorphic across the isolates used in the study. As an example, the data analysis for OPA-7 indicated that based upon the similarity matrix using the Jaccard coefficient, the 'white' and 'pink' isolates were 80 % similar to each other compared with 60 % between 'white' and 'plum' isolates (Table 4.10).

Table 4.10. Similarity matrix using the Jaccard coefficient.

	White	Pink	Plum	FotR1	FotR2	FotR3	FotR4
White	1	0.800	0.600	0.375	0.600	0.250	0.250
Pink		1	0.750	0.429	0.750	0.125	0.125
Plum			1	0.286	0.500	0.143	0.143
FotR1				1	0.286	0.222	0.222
FotR2					1	0.143	0.143
FotR3						1	1.000
FotR4							1

Using the same coefficient factor, the distance matrix could also be calculated (Table 4.11).

Table 4.11. Distance matrix using the Jaccard coefficient

	White	Pink	Plum	FotR1	FotR2	FotR3	FotR4
White	0	0.200	0.400	0.625	0.400	0.750	0.750
Pink		0	0.250	0.571	0.250	0.875	0.875
Plum			0	0.714	0.500	0.857	0.857
FotR1				0	0.714	0.778	0.778
FotR2					0	0.857	0.857
FotR3						0	0.000
FotR4							0

The cophenetic correlation coefficient was 0.97 and the relationship for the representative isolates based upon OPA-7 is shown in Figure 4.7.

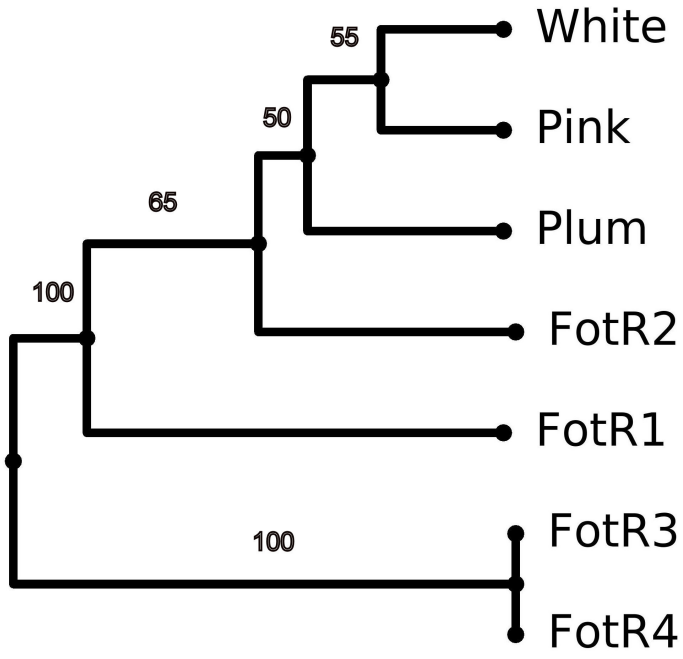
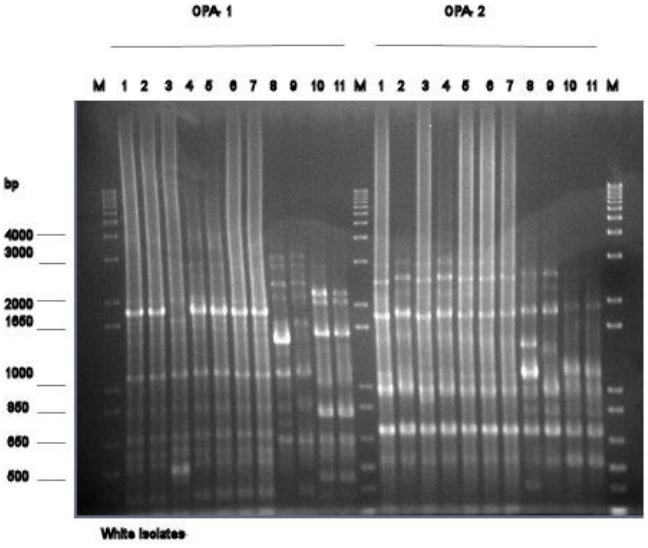


Figure 4.7. Dendrogram showing the relationship between the three NT *Fot* isolates and the reference *Fot* races 1 to 4 isolates. Bootstrap values are based upon 100 tree replicates.

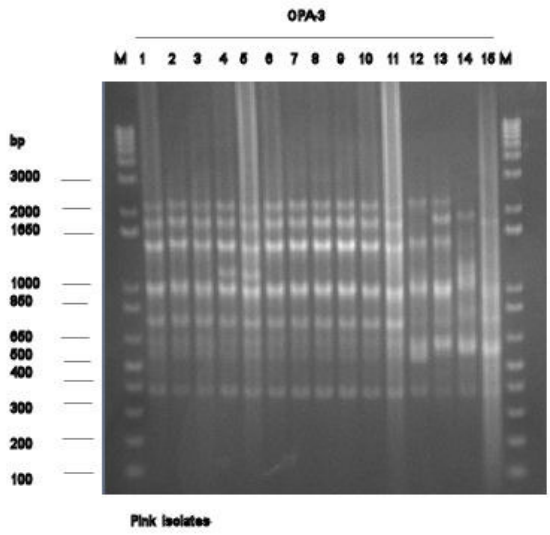
In almost all cases, the 'white' and 'pink' standard isolates were similar to each other and the 'plum' standard was similar to *Fot* race 2. These results indicated that white and pink may be more closely related to each other compared with the plum standard. The genetic diversity of the individual colour strains were investigated further using selected RAPD primers as identified in Table 4.2.

There was no genetic diversity observed in the group of 'white' isolates except for NTP-Dc34334 which had a slight different pattern using OPA-1 (Fig. 4.12a). All 'pink' isolates were identical except for NTP-Dc34325 and 34457 which were different using OPA-3 (Fig. 4.12b). All 'plum' isolates were identical except for NTP-Dc24947 which showed unique banding patterns with OPA-9 and OPA-15 (Fig. 4.12c). It should be noted that NTP-Dc34952 and 34383 showed similar pattern to the 'pink' isolates. Interestingly, only NTP-Dc34952 had a 'pink' colour resemblance but NTP-Dc34383 was more similar to the 'plum' isolate.

(a) White



(b) Pink



(c) Plum

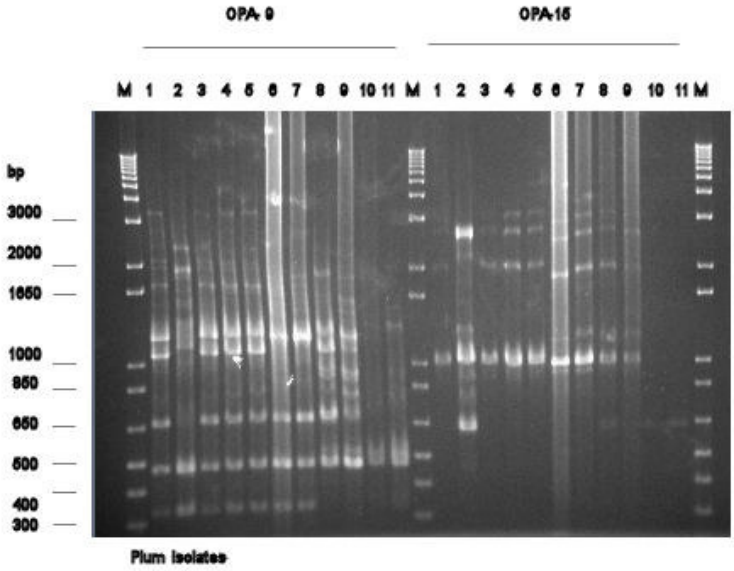


Figure 4.12. RAPD analyses for isolates within the three NT *Fot* strains. (a) 'white' isolates. M = 1 Kb Plus DNA ladder (Invitrogen, Australia), 1 = NTP-Dc26536 (standard), 2 = NTP-Dc34326, 3 = NTP-Dc34334, 4 = NTP-Dc34391, 5 = NTP-Dc34941, 6 = NTP-Dc34943, 7 = NTP-Dc34970, 8 = Fot race 1, 9 = Fot race 2, 10 = Fot race 3, 11 = Fot race 4. (b) 'pink' isolates. M = 1 Kb Plus DNA ladder (Invitrogen, Australia), 1 = NTP-Dc26571 (standard), 2 = NTP-Dc34308, 3 = NTP-Dc34322, 4 = NTP-Dc34325, 5 = NTP-Dc34457, 6 = NTP-Dc34506, 7 = NTP-Dc34517, 8 = NTP-Dc34946, 9 = NTP-Dc34950, 10 = NTP-Dc34965, 11 = NTP-Dc34969, 12 = Fot race 1, 13 = Fot race 2, 14 = Fot race

3, 15 = Fot race 4. (c) 'plum' isolates. M = 1 Kb Plus DNA ladder (Invitrogen, Australia), 1 = NTP-Dc24946 (standard), 2 = NTP-Dc249476, 3 = NTP-Dc24948, 4 = NTP-Dc24949, 5 = NTP-Dc24950, 6 = NTP-Dc34383, 7 = NTP-Dc34952, 8 = Fot race 1, 9 = Fot race 2, 10 = Fot race 3, 11 = Fot race 4.

Multigene characterisation

The histone 3 gene was not amplified from any of the *Fot* isolates used in this study. This indicated that the gene may not be present in *Fot*. The TEF1α and the β tubulin genes were amplified and sequence data indicated that all were 100 % similar to other *Fusarium oxysporum* on GenBank. It is intended that all sequences used in this study will be uploaded and deposited into GenBank.

4.4 Discussion

4.4.1 Effectiveness and uptake of grafting to manage *Fusarium* wilt of snakebeans in the Northern Territory

The on-farm field trials demonstrated the positive effect of grafting in instances where the level of *Fusarium* wilt is moderate to severe. In situations where the level *Fusarium* wilt is low such as Site 3, the advantage of grafting over seedlings is marginal, and may not be economically advantageous considering the extra inputs required for grafting over direct seeding. This is illustrated in Table 4.2 which shows that there was only a 15 % increase in yield for grafted plants over seedling plants at site 3, compared with 114 % increase in yield for grafted plants over seedling plants at site 2.

At the onset of the project many growers and some researchers were sceptical about the benefits of grafting. The farmer at site 2 now maintains he will not grow snake beans without grafting. He is currently grafting approximately 6000 plants per year. His brother grafted a further 2-3000 plants in 2010. In spite of this the uptake of the grafting technology in the snake bean industry in Darwin has been slower than initially expected. This is likely due to an interaction of many factors, the most significant of these being the need to transplant grafted seedling in an industry currently based on direct sown snake bean seeds. This requirement to produce transplants, the additional infrastructure (eg. mist tent or seedling nursery) and labour associated with it is a significant deterrent to many growers used to simply sowing the seed directly into field soil.

Although grafting is advantageous in all situations where snake bean *Fusarium* wilt occurs, it is probably only economically advantageous where the level of *Fusarium* wilt is moderate to severe, not where the level of *Fusarium* wilt is low. Low, medium and moderate are difficult to define in terms of percent infection. For instance, at trial site 3 with "low levels" of inoculum, infection was at 80 %, 140 DAP (Fig. 4.1). However, the first infection did not occur at this site until 71 DAP. By contrast, all plants were dead at Site 1 by 78 DAP.

4.4.2 Isolate characterisation/race determination

This present study represents the most extensive study of Fot isolates outside the USA. The only other previous reports into race characterization outside the USA were those of Armstrong and Armstrong's 1980 study of an isolate from Nigeria as Race 1, and the unpublished 1999 report of Professor Yi-Sheng Lin, Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan, R.O.C., of Race 3 in Taiwan.

The race differential work in this study shows that the white and pink strains are two new races, on the basis of Patel-Ehlers differentials. It is unlikely that these will be separated except with extra differentials. The Plum strain is identical to Race 2 on the Patel-Ehlers' differentials. However, infection of Mississippi Silver (5/15, 3/15) suggests that even though it may be close to Race 2, it may not be identical.

The 'white' strain was more aggressive on most cowpeas and also in infecting GPK snake beans (Tables 4.6, 4.7, 4.8 and 4.9). However, the 'pink' strain was isolated in far greater numbers than the 'white' or 'plum' strains from *Fusarium* wilt affected snake beans since 1999, including from field samples in 2009. This suggests that the 'pink' strain must have other factors that affect its prevalence as the dominant strain in GPK snake bean plants in the field situation.

Haglund and Kraft (1979) documented the occurrence of a new race 6 of *Fusarium oxysporum* f.sp. *pisi*, emphasising the need to use cultivars with resistance determined by "single separate dominant genes in the host". Race differentials may or may not have single dominant genes, however their resistance is vertical or qualitative, not horizontal or quantitative, determined by "major genes" not "minor genes" (Keane and Brown 1997). Not only is Iron resistant to the

four races in USA, it is also resistant to the three strains of Fot in Darwin. This indicates that Iron has an array of "R genes" (Agrios 2005). TVu 347 in Armstrong and Armstrong (1980) study was resistant to races 1 and 2, but susceptible to race 3. In our work, we found TVu 347 resistant to all our strains. This suggests that TVu 347 has numerous "R genes", and can be used as a susceptible indicator of Race 3.

In some instances, considerable variability in pathogenicity was recorded in this study, eg, the reactions of 13/15 and 3/15 for inoculation of PI 115681 with the 'white' strain and 8/15 and 4/15 (Table 4.6) for inoculation of PI 162925 with the 'pink' strain (Table 4.8). This reinforces the need for the race screening work to be repeated with larger numbers of plants (e.g., 4 x 8 = 32 plants) as suggested by Haglund and Kraft (1979), in order to confirm the results detailed here. Armstrong and Armstrong (1980) used plant numbers varying from 8 to 104 in their investigation of race 1 from Nigeria. They also gave the plants a second inoculation 5-7 days after the first inoculation. We usually also gave our plants a second inoculation. This was not done here because of a lack of available time.

The race screening work recorded in Table 4.9 indicated several additional sources of resistance to the three strains of Fot found in the Northern Territory. These are Giant (from Greenpatch Seeds, Taree), Ebony, Meringa, TVu 401 (AusTRCF 84809 accession), NT 2223, NT 2436, and NT 5473. These together with previously identified resistant lines could be a source of resistance should a breeding program be initiated to produce a resistant snake bean. Another possibility is searching for survivors in *Fusarium* wilt affected farms and screening the progeny to verify resistance to Fot. This technique was how CB3 was selected from surviving CB 5 plants in the Chino district of California (Smith 1948). As well as Iron, other cowpea lines such as PI 166146 (originally collected from India) and CB 3 have been used in breeding for resistance to *Fusarium* wilt in cowpeas in California (Helms *et al* 1991a; Helms *et al* 1991b; Ehlers *et al* 2000). Most of the TVu lines including TVu 401 were obtained previously to investigate a cowpea virus. The Northern Territory Department of Resources has two AusTRCF accessions of TVu 401, AusTRCF 84809 and AusTRCF 84664. The AusTRCF 84809 accession tested as resistant, whilst the AusTRCF 84664 accession tested as susceptible to all three Darwin strains (Table 4.9). These two AusTRCF accessions previously gave different reactions to the cowpea virus.

4.4.3 Molecular characterisation

Several techniques were used in this study to characterise the genetic relatedness of the three NT *Fot* standard ('white', 'pink' and 'plum') isolates against the four known *Fot* races. Several efforts were made to obtain the newly described strain from *Gerbera jamesonii* for inclusion in this study with no success.

Although there has been substantial amount of information known about the Fusarium genus, not much genetic information is known about *Fot*. To date, molecular characterisation of *Fot* is limited to several nucleotide sequences on GenBank (http://www.ncbi.nlm.nih.gov/). Several genes were characterised within this study and will be uploaded onto GenBank to expand the current genetic knowledge for this organism. This study showed that the histone 3 could not be amplified from the *Fot* isolates. To the author's knowledge, this has not been documented before.

Genetic characterisation of *Fot* is essential to understand the organism. This study demonstrated the usefulness of using genetic data and various fingerprinting techniques to provide valuable information to variability and genetic relatedness of the three NT isolates compared with the four reference races.

In this study, the RFLP analysis of the IGS region was used to examine the relationship between the three standard isolates compared with the four known races. Using the CNL12 and CNS PCR primers, a 2.6 Kb PCR product was amplified covering the IGS region. This was also demonstrated in previous studies of *F. oxysporum* f.sp. *lycopersici* (Cai *et al.* 2003); *F. avenaeum*, *F. arthroporioides* and *F. tricinctum* (Yli-Mattila *et al.* 2002) and *F. oxysporum* f. sp. *fragariae* (Nagarajan *et al.* 2004). The RFLP analysis of the *Fot* isolates in this study using four restriction enzymes indicate that the white and pink isolates were genetically indistinguishable but polymorphism was observed with the plum isolate. Interestingly, *Fot* race 3 could not be amplified using the CNL12 and CNS primers although no PCR inhibitors were found, since no difficulties occurred for other PCR tests.

RAPD analysis was used to determine genetic relatedness between the three NT standards compared to the known four *Fot* races. Initial analysis based upon 20 decamers, some of which were eliminated from further studies since no diversity was observed or the amplification did not provide clear distinguishable bands suitable for analyses. Of the 20 RAPD primers, ten was used to further characterise several isolates within each colour group. Results indicate that the NT strains generally clustered with the *Fot* races 1 and 2 using the UPGMA method. Patterns showed that the 'white' and 'pink' strains were most likely closely related compared to the 'plum' strain.

Several techniques used within this study indicate that although the plum strain is closely related to the *Fot* race 2, there was no evidence that any of the three were genetically similar to any of the four known races. Genetic data using multigenes did not allow characterisation beyond species level. Therefore RFLP and RAPD data was used to determine

relatedness. Various genetic marker systems were used to determine genetic diversity among different isolates within a species, particularly *F. oxysporum* f. sp. *cubense* which is a haploid asexual pathogen (Bentley *et al.* 1998). Although the VCG of *Fot* is not known, its usefulness in genetic diversity studies is questionable since there was little or no variation observed among isolates within each VCG group of *F. oxysporum* f. sp. *cubense*, irrespective of geographic location or host. Although the coloured strains were found on different locations in this study, there was no genetic diversity observed among the isolates. A parallel race differential study on the three NT *Fot* isolates is currently being conducted. The resulting information from that study should provide some insight into the possible race origins of the three NT *Fot* isolates and provide supporting evidence to the molecular studies described in this report.

4.5 Grafting Review – The potential of grafting for soilborne disease management in Australian vegetable production systems

4.5.1 Summary

This study reviewed the potential and feasibility of the use of grafting of resistant rootstocks for soilborne disease management in Australian vegetable production. In some regions of the world there has been an expansion of the use of grafting resistant rootstocks over the last 10-20 years due to restrictions in the use of soil fumigants such as methyl bromide and an interest in biological production systems. In Australia recent studies on a *Pythium-Fusarium* disease complex in cucumber and a *Fusarium* wilt of snakebeans have reported good disease control using grafting.

A number of other crop-pathogen-resistant rootstock systems for economically important pathogens in Australia have been evaluated overseas and may be suitable for commercial use in Australia. These included the use of resistant root stocks for managing: *Fusarium* wilt in cucumbers; rootknot nematodes in capsicum and eggplant. A number of other soilborne disease grafting systems were reported for non-levied vegetable crops including tomatoes, melons and watermelons. The use of grafting systems would require evaluation and adaptation for use in Australian conditions and with the specific vegetable cultivars of importance in Australia. It was noted overseas that conditions of high soil temperatures provide an advantage to some pathogens even when tolerant rootstocks are used. This area may require particular attention when evaluating efficacy and effects of environmental conditions. Grafting may also be of potential use in the management of soilborne pathogens in Australia that have not had resistant stock developed or evaluated overseas, however, progress in this area would require fundamental research in Australia.

Grafted seedlings are more expensive than non-grafted, a favourable cost:benefit balance is therefore required to warrant their use. Therefore it is important that the rootstocks used in the grafting process be selected where possible with resistance to more than one soilborne pathogen. A number of additional potential benefits of grafting have been reported including higher yields, more stable yields, improved fruit quality, early or late season production capability, and tolerance to particular abiotic conditions such as soil salinity. It is important that the effectiveness of these characteristics is evaluated under Australian conditions that any additional potential benefits can be included in the cost:benefit analysis. Due to the costs of grafting, rootstocks should be selected that seek to manage problem pathogens and also provide additional production benefits. In addition, the relative cost of grafting differs between production systems, the relative cost of using grafting may be lower in high input or setup systems such as protected cover or trellised systems that use long production cycles with multiple picks. In this scenario the improved production and disease management provided by grafting of elite rootstocks would be expected to offset the grafting cost at a lower threshold than for single pick and low cost structured field production systems.

Two cases of the use of grafting for disease management in tomatoes, a transplanted crop, and snakebeans, a direct sown crop, in Australia indicted that uptake by growers may be higher for crop production systems where transplants are used. In addition for pathogens with inoculum that is widespread and well established, such as for *Ralstonia solanacearum* in the Darwin region, grafting may be one of the only options for tomato production in soil. In contrast for relatively newly arrived and dispersing pathogens, such as *Fusarium oxysporum* f.sp. *tracheiphilum* in snakebean, growers may seek to shift production to avoid the pathogen and avoid the extra labour and investment required for production of resistant grafted seedlings.

In order for growers to make informed assessments of the need and value of the use of grafted seedlings at a site for a particular crop, the identification and development of information or services on a number of aspects in required. These aspects are the:

1. identification of rootstocks available in Australia that provide consistent levels of disease resistance.
2. identification of disease resistant stocks that also have dual production advantages such as particular abiotic tolerances, late or early season production capability, production of specialist fruit quality, or have high production levels.
3. development of inoculum quantification tools for site assessments for important pathogens linked to inoculum-management based disease risk assessments.

4. economic information on a) the disease risk at which grafting will be a profitable management option b) the level of improved production or return from dual purpose rootstocks at which grafting will be a profitable management option including comparison of multiple pick versus single pick crops, and c) analyses of the comparative cost of shifting production to clean land for broadacre production of vegetables in comparison to the use of grafted transplants at an existing infested site.

Companies providing transplants to growers who also provide high quality crop specific grafting services will also be required for use of this strategy on a substantial scale.

4.5.2 Introduction

This review focuses on the potential and feasibility for the use of grafting for soilborne disease management in the specific context of the Australian vegetable production. A number of reviews have covered the history and technological advances in the grafting of resistant rootstocks for disease management in vegetable production outside of Australia, with Lee and Oda (2003) providing a comprehensive review of this topic. Later reviews have dealt with selected vegetable crops, topics or regions (Lee and Oda 2003; Cohen *et al.* 2007; Oda 2007; Davis *et al.* 2008b; Sakata *et al.* 2008b). This review does not seek to repeat this work and focuses on the potential and feasibility for use of grafting for soilborne disease management in the context Australian vegetable production. Prior to this grafting for disease management in Australia appears only to have been evaluated for a small number of crops and specific pathogens without consideration for wider use.

The principal focus of the review is the potential to manage four genera of soilborne fungal pathogens (*Sclerotinia, Rhizoctonia*, *Pythium* and *Fusarium*) which were identified as the major soilborne pathogens limiting vegetable production in Australia (Donald *et al.* 2010). The use of grafting as a disease management tool is best suited to high value crops that produce several units per plant and may include multiple harvest dates. The review focuses on the following high value vegetable fruit crops: capsicum, tomato, beans, watermelon and other melons, cucumber and eggplant.

In evaluating the feasibility of grafting for use in Australia, attention was directed to identifying practical, economic and technical limitations to implementation and uptake. An important aspect of grafting is that some resistant rootstocks can provide management of more than one pathogen (Ioannou 2001; Crino *et al.* 2007; Wu *et al.* 2008b), or provide improved environmental tolerance to limiting temperatures, flooding, drought or salinity (Lee 1994) and/or improved yield or quality characteristics (Lee 1994; Davis *et al.* 2008a). Because of the wider potential benefits of grafting, studies that also cited improved management of other fungal pathogens, bacteria and nematodes in addition to improved environmental tolerance or yield attributes were also included.

4.5.3 Use of grafting

The limited availability of land is cited as one of the reasons for adoption of grafting (Lee 1994; Taylor *et al.* 2008). In regions with high populations, intensive horticulture and crop rotations, and limited land area, the management of soilborne pathogens is difficult particularly when production cannot be shifted to new pathogen free land or if chemical controls are not available or inadequate. Soilborne pathogens that have a wide host range such as *Fusarium oxysporum,* which can persist as a saprophyte on non-hosts in the absence of a susceptible host, or produce durable resting structures such as chlamydospores are particularly difficult to control.

Historically, the extensive use of grafting has been associated with particular regions such as Asia (Lee 1994; Davis *et al.* 2008b; Sakata *et al.* 2008b). For example, in 1994 35,100 and 37,800 ha of field grown watermelon, cucumber, oriental melon, muskmelons, tomato and eggplant were produced from grafted seedlings in Japan and Korea respectively, the respective areas under green houses for these crops were 14,100 and 19,300 ha (Lee 1994). By 1998 95 % of watermelon grown in Japan, Korea and Taiwan were from grafted seedlings (Lee *et al.* 1998). Although some areas of Europe have made use of grafting to manage soilborne cucurbit pathogens for approximately 50 years (Koutsika-Sotiriou and Traka-Mavrona 2002; Cohen *et al.* 2007), extensive use of grafting to manage soilborne pathogens has only received attention over the last decade in areas of Europe (Bletsos *et al.* 2002; Amadio 2005; Bletsos 2006) and North America (Davis *et al.* 2008b; Taylor *et al.* 2008) due to efforts to replace particular soil fumigants or for use in biological farming (Rivard *et al.* 2008). In Australia similar issues influence the contemporary management of soilborne pathogens (Donald *et al.* 2010).

This section introduces and discusses a number of important aspects of grafting including the level of pathogen management, rootstock:scion compatibility, grafting and after-care methods, new technologies and the capability of grafting to contribute to the management of other (non-soilborne) pathogens, abiotic stresses and improved production.

Extent of soilborne pathogen management provided by grafting

The level of management provided by a rootstock is generally related to its degree of resistance or tolerance and whether the scion was susceptible to infection through adventitious root growth or soil contact.

Success of grafting practices on controlling diseases: The level of pathogen management through grafting can be extremely high as shown in studies of Fusarium wilt (Table 4.13). For example in rootstock field trials of a number of species and hybrids for resistance to *Fusarium oxysporum* f. sp. *niveum*, no wilt symptoms were observed in all trialled rootstocks except the susceptible control (Yetisir *et al.* 2003). In other field studies infection of scions on resistant stocks can be recorded after nil infection of the stock was observed in laboratory trials. In some studies this infection of the scion was thought to be due to soil splash or growth of adventitious roots from the graft union (Pavlou *et al.* 2002). The lower disease levels in grafted plants can have large effects on productivity. Over eight years watermelon grafted to *Fusarium* wilt resistant stock had a 2.3 fold higher plant survival and a 3.2 fold higher yield (Miguel *et al.* 2004).

Table 4.13. Examples of the level of *Fusarium* wilt control using grafted rootstocks from three recent studies.

Pathogen	Crop	Rootstock	Level of disease management relative to control	Source
Fusarium oxysporum f. sp. *radicus-cucumericum*	Cucumber	*Curcubita ficifolia*, *C. moschata* and *C. maxima x C. moschata* hybrids	Control had 97 % incidence, best stocks 8 and 13 % incidence, infection thought to be from adventitious roots from graft union	(Pavlou *et al.* 2002).
Fusarium oxysporum f. sp. *niveum*	Watermelon	*Cucurbita moschate, C. maxima, Lagenaria siceraria*, *Luffa cylindrica*, *Benincasa hispida*, *Lagenaria* hybrids and *Cucurbita* hybrids	The control had 5-10 % wilt, grafted plants had nil wilt	(Yetisir *et al.* 2003)
Fusarium oxysporum f. sp. *lycopersici*	Tomato	Resistant Tomato	Control had 48 % incidence, two resistant stocks had 0 and 29 % incidence	(Rivard and Louws 2008)

Resistance or tolerance to additional soilborne pathogens: The use of resistant rootstocks to manage a target pathogen can also have additional effects on other soilborne pathogens, in fact for some rootstocks multiple resistance or tolerance to an additional soilborne pathogen can be quite common. For example, a review of rootstocks used in tomato production cited 10 studies of rootstocks for soilborne disease management; four of these studies reported multiple disease resistance. For example, the rootstock tomato 'Magnet' has two resistance genes to Tobacco mosaic virus (TMV) and is highly resistant to *Pyrenochaeta lycopersici*, *Fusarium oxysporum* f.sp. *lycopersici* race 2, *Verticilium dahliae*, and *Ralstonia solanacearum* [formally *Pseudomonas solanacearum*] and has tolerance to *Fusarium oxysporum* f.sp. *lycopersici* race 1, and nematodes (Lee and Oda 2003). Similarly, a number of watermelon rootstocks have resistance to either two or three pathogens, namely *Colletotrichum orbiculare*, *Diplodia bryoniae*, and *Fusarium oxysporum* f.sp. *niveum* race 0 or race 1 (Lee and Oda 2003). In a recent field study in the United States, intraspecific tomato rootstocks also provided high levels of control of *Ralstonia solanacearum* and Tomato Spotted Mosaic Virus (TSMV) in addition to the primary target pathogen, *Fusarium oxysporum* f.sp. *lycopersici* (Rivard and Louws 2008). Appendix 4.1 (Table 4.18) lists a number of recent grafting studies, nine of these studies reported multiple resistance or additional tolerance to other soilborne pathogens.

Resistant vs. tolerant rootstock: The use of stocks that are tolerant as opposed to resistant to pathogens has been observed to provide variable results. Tolerance is here defined as the ability of a particular species or cultivar to yield despite infection. The level of disease control achieved using stocks that are tolerant to the target pathogen is usually not as high as that of resistant stocks, or tolerant stocks can provide less consistent control due to environmental factors or high inoculum pressure severely challenging tolerance (Cohen *et al.* 2007; Davis *et al.* 2008b). Some rootstocks for use with cucurbits are resistant to *Fusarium* wilts such as *Fusarium oxysporum* f. sp *niveum* in watermelon, but stocks for *Monosporascus cannonballus*, *Phytophthora capsici* and *Verticillium dahliae* management are tolerant to these diseases (Davis *et al*. 2008b). The use of stocks to manage nematodes is another useful example as genetic resistance to nematodes such as *Meloidogyne* spp. is not reported but accessions that are tolerant have been identified (Cohen *et al.* 2007; Huitron *et al.* 2007).

High soil temperatures or high inoculum concentrations provide advantages to some pathogens with the use of tolerant rootstocks. For example, rootstocks of a tomato provided management of *Meloidogyne* spp. in winter, but not during summer when soil temperatures were high (Ioannou 2001). Management of *Meloidogyne* spp. with tolerant tomato

stocks was reported as effective as long as the soil temperature does not exceed 27°C or 32°C for some varieties (Messiaen 1995, cited by (Miguel 2005b)). In other studies on *Meloidogyne incognita* (root knot nematode) management using tomato stocks, adequate levels of management were only provided using rootstocks when the density of galls was not high (Lopez-Perez *et al.* 2006). However, more recently five wild watermelon lines and one commercial line were shown to be highly tolerant to infection by *M. incognita* (Thies *et al.* 2010). The authors have claimed these stocks to be resistant to *M. incognita* but more work is required to confirm this. Where stocks are tolerant as opposed to resistant the inclusion of additional cultural practices or pesticides may be required to attain suitable levels of control, especially at sites where inoculum levels are high or soil temperatures favour a rapid increase in the pathogen population.

Integrated grafting practices: To manage some pathogens the use of rootstocks in combination with additional practices may be required. The use of rootstocks in combination with pesticides has given high levels of efficacy in some crops. Some examples are: management of *Colletotrichum coccodes* at medium to high incidence levels in tomato using a combination of tolerant tomato rootstocks and dimethyl disulfide at 40 or 80 g m^{-2} or metham sodium at 192 g m^{-2} (Garibaldi *et al.* 2008); and management of *M. incognita* in greenhouse cucumber using *Fusarium* wilt tolerant stocks (*C. maxima* x *C. moschuta*) in conjunction with dazomet (Giannakou and Karpouzas 2003).

Some rootstocks can provide adequate control against particular pathogens and partial control against others, but integration with an additional cultural practice may provide high overall levels of control. For example, grafting of resistant tomato rootstock ('Brigeor F1') on eggplant provided complete protection against *Pyrenochaeta lycopersici*, but protection from *Meloidogyne* spp. only occurred in trials with greenhouse-grown (winter) crops or open-field (summer) crops with about 50 % of the grafted plants sustaining slight nematode infection and partial protection from *Verticillium dahliae* (Ioannou 2001). But in combination with soil solarisation grafting provided almost complete control of the three pathogens. Soil solarisation alone was highly effective against *Verticillium* wilt but only partially effective against *Pyrenochaeta lycopersici* and *Meloidogyne* spp.

Disease complexes and grafting: A number of important diseases of vegetables in Australia are caused by disease complexes (Stirling *et al.* 2004a; Tesoriero 2009). Most pathogen management grafting systems are typically targeted towards the management of a specific production limiting disease such as *Fusarium* wilt in tomatoes and watermelon (Lee and Oda 2003; Keinath *et al.* 2010). Although, as previously noted, a substantial number of rootstocks also provide additional resistance or tolerance to a number of other pathogens, in these cases grafting is assisting in the management of multiple pathogens that act to cause separate diseases, not as disease complexes. The management of disease complexes with grafting appears to have received little attention, with one important exception being the study of Tesoriero (2009) on wilt complex in greenhouse cucumber caused by *Pythium aphanidermatum* and *Fusarium oxysporum f.sp. cucumerinum*. Cucumber grafted on to the cucumber stock 'Ferro' provided high levels of control (90-100 % plant survival) after inoculation with both pathogens separately, ungrafted plants had a higher death rate. Although the pathogens were not applied as a combined mixture, the results from the separate treatments indicated that there may be potential to manage some disease complexes with the use of grafting. Further work is required in this area.

Specialised production systems: Grafting may be used to provide an additional management tool in some production systems such as certified organic or low input systems where alternative curative management options as opposed to preventative methods may be limited. The use of grafting in certified organic productions systems has been demonstrated to provide an effective practice for vegetable production at sites with soilborne pathogen problems in the USA (Rivard and Louws 2008; Rivard *et al.* 2008) and low input systems in Europe (Curuk *et al.* 2009).

Importance of rootstock:scion compatibility

The literature indicates that although many resistant rootstocks can protect the scion from infection by soilborne pathogens, rootstock compatibility is very important to ensure that production levels and quality of product from the scion is as good or better than from the non-grafted scion. Incompatibility differs from graft failure, incompatibility occurs despite a successful graft and may be the result of a weak graft union or physiological incompatibility due to factors such as lack of cellular recognition, wounding responses, effects of growth regulators or incompatibility toxins (Davis *et al.* 2008b). Incompatibility may be visible at the graft union in terms of visible overgrowth or undergrowth or a poor vascular connection, but generally incompatibility may be less evident due to physiological incompatibility resulting in: growth suppression (Huh *et al.* 2003); reduced yields (Yetisir *et al.* 2003); reduced fruit quality (Davis *et al.* 2008a). For example, *Cucurbita moschuta, Cucurbita maxima*, *Cucurbita* hybrids, *Luffa cylindrica*, *Benincasa hispida*, *Lagenaria siceracia* and *Lagenaria* hybrids stocks all provided high levels of disease resistance to *Fusarium oxysporum* f.sp. *niveum* (races, 0, 1 and 2) in watermelon, but *Cucurbita* stocks provide lower quality fruit and lower yields than watermelons grafted on *Lagenaria siceracia* and *Lagenaria* hybrid rootstocks (Yetisir *et al.* 2003).

Incompatibility effects appear complex and are often rootstock:scion specific especially for effects on produce quality. Non-grafted eggplants were less sweet than those grafted onto *Solanum torvum* and *S. sisymbriifolium* rootstocks to

protect against *Verticillium dahliae* infection (Arvanitoyannis *et al.* 2005) and water melon rootstocks gave better water melon fruit colour than fruit from bottlegourd rootstocks (Huh *et al.* 2003). There was a fruit quality benefit in watermelon for the cut melon market, as melons grafted onto *Fusarium* wilt resistant stocks of *Cucurbita* or *Lagenaria* were firmer after cutting than non-grafted water melon and also stayed firmer in storage after cutting than fruit from non-grafted plants (Taylor *et al.* 2006). The crisper fruit from grafted plants was identified as being important for the fresh-cut watermelon market (Taylor *et al.* 2006). However, in some cases grafting has no effect on produce quality (Miguel *et al.* 2004; Sakata *et al.* 2008a) or produce quality can be both positively and negatively affected by specific rootstock:scion combinations (Davis *et al.* 2008a).

The key message regarding incompatibility is that a disease resistant stock can protect the plant from soilborne pathogen infection but may not provide the best yield or quality of production, therefore, a range of resistant stocks should be trialed with the particular cultivars of scions to be used.

Grafting and after-care methods

A range of grafting methods have been developed as suitable for soilborne pathogen management and suitable for particular crops. This section provides an overview of popular methods, and a basis for selection of grafting and after-care methods.

In some cases grafting methods have been developed for or are particularly successful for specific rootstock:scion combinations (Oda 1995) such as hole insertion grafting for watermelon (Davis *et al.* 2008b). The method of grafting used for specific crops can be important to the success of the procedure, for example, the use of a grafting method used for tomato on cucumbers gave seedlings which had some problems with nutrient transport (Tesoriero 2009). Table 4.14 provides an overview of popular grafting methods.

Table 4.14. Overview of grafting methods and their associated advantages and disadvantages.

Method	Advantages	Disadvantages	Described by
Tongue approach or approach graft	Easy to learn and requires a low level of humidity after grafting.	Requires purchase of clips and labour to remove clips 15-20 days after grafting	(Lee 1994; Davis *et al.* 2008b)
Hole insertion or Terminal or Top insertion graft	Described as easy to perform in cucurbits. High graft union provides better disease protection. Used for capsicums and cucurbits	The size of the rootstock hole is limited by rootstock size	(Lee 1994; Davis *et al.* 2008b; M'Hamdi *et al.* 2010)
One cotyledon, Slant, Splice or Tube grafting	Suitable for stocks that have thin stems eg. watermelons and cucumbers.	Requires good post-graft management to protect scion junction	(Davis *et al.* 2008b)
Cleft or Side insertion graft	Suitable for seedlings with wide hypocotyls. Reported to have a higher success rate than the above 3 methods.	Requires a clip.	(Lee 1994; Davis *et al.* 2008b)
Pin graft	Pin maintains good graft-scion contact	Scion and rootstock need to be the same diameter for close cambial contact	(Davis *et al.* 2008b)
Double graft	Used to bridge non-matching sized stock and scion	Requires additional labour	(Davis *et al.* 2008b)
Root pruning	An additional step to some of the above methods where the stock's hypocotyl is cut to remove the roots to increase production of primary roots. Popular in Japan for watermelon.	Requires additional work to the above methods.	(Davis *et al.* 2008b)
Automated grafting	High volume of transplants per hour	Requires specialised machinery and operators	(Kurata 1994; Lee and Oda 2003; Davis *et al.* 2008b; Kubota *et al.* 2008)

Although many methods have been described and are recommended for particular crops, such as a success rate of 88 % for side grafting of eggplant onto *Solanum torvum* and 80 % for approach grafting in melon (Bletsos *et al.* 2002), there appears to be limited information comparing grafting methods to identify which methods for which crops provide the highest percentage of successful grafts. One exception is that cleft grafting was less successful than tongue approach grafting in cucumber in Japan (Sakata *et al.* 2008b). Some grafting methods may have additional advantages, for example for tomato, although hole insertion grafting can be quite slow and the success rate lower than other methods the graft union fusion is stronger than tongue approach grafting (Lee and Oda 2003). These aspects can be important for the survival of grafted seedlings in challenging conditions, such as for watermelon seedlings in windy areas where grafting methods incorporated a modified tube or a toothpick used as a splint to protect grafts and provide improved seedling survival after transplanting in windy areas (King and Davis 2006).

After graft healing facilities can be very important to achieve an acceptable level of successful grafts using some methods such as the 'one cotyledon' method (Davis *et al.* 2008b). After care acclimatization facilities typically require high humidity levels, reduced light and efficient irrigation. Details of acclimatization facilities and designs were summarised by Lee and Oda (2003), they noted that the sealing of cell trays (50 or 72 cells) with single layer semitransparent 0.01 mm high density polyethylene film reduced moisture losses and additional irrigation requirements.

New technologies

Some new technologies are under investigation, the success of these investigations may be important to increase the range of pathogens or efficacy of soilborne pathogen management by grafting. For example modified rootstocks may extend resistance ranges, the use of transgenic rootstocks with genes that code for the putative 54-kDa replicase gene of Cucumber Fruit Mottle Mosaic Tobamovirus (CFMMV) protected susceptible cucumber scions from infection through CFMMV infested soil (Gal-On *et al.* 2005). Molecular methods can also be used to select rootstocks with resistance, for example, lines of bottle gourd *Lagenaria siceraria* (bottle gourd) were sequenced and screened for resistance to *M.*

incognita, Zucchini Yellow Mosaic Virus and tolerance to powdery mildew resistance in order to identify genes for use in selecting stocks for watermelon (Levi *et al.* 2009).

Management of diseases other than soilborne pathogens

Whilst the focus of this study is on soilborne pathogens, a number of soilborne pathogen resistant rootstocks also provide some level of resistance or tolerance to non-soilborne pathogens. Where grafting may assist in the management of multiple pathogens it is important to consider cumulative benefits that may accrue, as these may increase the ratio of benefit:cost. For example, some pathogens which are plant residue borne such as *Didymella bryoniae* are managed by the grafting of stocks resistant to *Fusarium oxysporum* f. sp. *melonis* in melons (Crino *et al.* 2007). Similarly a large number of rootstocks used for watermelon also have resistance to *Colletotrichum orbiculare*, another pathogen that survives on crop residues (Lee and Oda 2003). The replacement of *C. maxima* x *C. moschuta* and *C. maxima* rootstocks for cucumber in Japan with *C. moschuta cv*. Kitora was associated with an increased susceptibility to *Podosphaera xanthi* (Powdery mildew) and *Corynespora cassicola* (Corynespora leaf spot) (Sakata *et al.* 2008b). It may be useful to select rootstocks that have additional non-soilborne pathogen management roles.

Non-disease management benefits from grafting

A large number of studies reviewed by Davies *et al.* (2008b) and Lee & Oda (2003) cite non-disease management benefits of grafted cucurbits that included enhanced tolerance to abiotic stresses such as cold temperatures, flooding, drought and salinity, increased yields associated with increased vigour or improved nutrient or water use efficiency. In some cases where abiotic conditions were limiting to non-grafted plants, grafted plants also had extended harvest periods. However, what is important from a pathogen management perspective is that some stocks that are resistant to soilborne pathogens can, in addition to this pathogen protection role, also confer some of the above non-disease management benefits.

Soilborne pathogen resistant rootstocks have been found to confer a number of different non-disease management benefits. Tomatoes with eggplant stocks for *Ralstonia solanacearum* management produced fruit for a longer period due to the resistant stocks also being selected for flood tolerance (Palada and Wu 2007). Other examples include: salt tolerance also reported for *Phythophthora drechsleri* tolerant bottlegourd stocks used in watermelon grafting (Chung *et al.* 2003); pathogen and drought tolerance in muskmelon (Jifon *et al.* 2007). Where multiple pathogen and non-pathogen management benefits may be gained through the use of grafting then the cost:benefit ratio may be more favourable due to additional non-disease related economic benefits.

Two major transplant supply companies were contacted and asked if they were providing grafted seedlings for use in soilborne pathogen management. A Victorian seedlings supplier suppling grafted tomato seedlings using 'Maxifort' rootstock, stated that the stock was being used for plant strength and vigour rather than for soilborne disease management reasons (M. Henshall, pers comm. Oct. 2010). Some trials of grafting in watermelon, tomato and capsicum have been carried out by seedling supply companies in Queensland, but the practice is not used commercially (E. Jovicich, pers comm. Oct. 2010).

Soilborne pathogens in Australian crops managed with grafting

In Australia several studies have investigated the use of grafting to manage soilborne diseases in vegetable crops and the techniques developed have been used by growers in various regions (Table 4.15). Studies evaluated grafting to manage: *Ralstonia solanacearum* of eggplant in Queensland (Gordon 1994) and tomato in the Northern Territory (Poffley 2003b); and a disease complex caused by *Pythium aphanidermatum* and *Fusarium oxysporum f.sp. cucumerinum* in cucumber in New South Wales (Tesoriero 2009).

Two recent studies have highlighted that grafting can be used to achieve high levels of pathogen management (Tesoriero 2009; Donald *et al.* 2010) that also resulted in significant increases in production for growers (Conde *et al.* 2010b; Donald *et al.* 2010). Grafting has also been an economically important practice for management of *Ralstonia solanacearum* in soil grown tomatoes produced in Darwin in the Northern Territory over approximately a twenty year period (Poffley 2003b). The use of grafted snakebeans to manage *Fusarium oxysporum* f.sp. *tracheiphilum* and grafted tomato to manage *Ralstonia solanacearum* in the Northern Territory were selected as case studies in order to identify components that contributed to the success of the practice in terms of adoption by commercial producers.

Table 4.15. Research on grafting for controlling soilborne pathogens in Australian vegetable crops.

Crop	Pathogen	Grafting on	Citation
Beans (Snake)	*Fusarium oxysporum* f.sp. tracheiphilum*	Cowpea	(Conde *et al.* 2010b)
Cucumber	*Pythium aphanidermatum* and *Fusarium oxysporum f.sp. cucumerinum* complex	Cucumber	(Tesoriero 2009)
Eggplant	*Ralstonia solanacearum** *[Pseudomonas solanacearum]*	*Solanum torvum*	(Gordon 1994)
Tomato	*Ralstonia solanacearum** *[Pseudomonas solanacearum]*	*Solanum melongena* var. wild Malay and *S. torvum*	(Poffley 2003b)

*Growers were known to have used this grafting practice following these research projects

4.5.4 Case study 1: Tomato grafting in the Northern Territory

Ralstonia solanacearum was described as widespread in occurrence on red lateritic soils of the northern coasts of the Northern Territory and as serious disease of tomatoes in these areas (Heaton and Benson 1968; Scholefield and Blackburn 1985), the earliest documented identification of the pathogen in the area was in 1961 (Morschel 1961). In later work the pathogen was associated with a wilt of *Stylosanthes humilis* which occurred at sites up to 60 miles south of Darwin and in sites where the land had never been cultivated or cropped (Aldrick 1971). Due to the prevalence of *Ralstonia solanacearum* on uncropped land Aldrick (1971) proposed that the pathogen could be indigenous to the area unless it had been introduced on seed.

In 1979 a research program was established in the Northern Territory to select tomato lines for resistance to *R. solanacearum* which causes bacterial wilt. Resistance was required under high soil temperature conditions, and fruit size, quality and ability to set fruit in hot conditions were the production traits required. In addition selections that set fruit in hot conditions were required for grafting onto resistant stocks (Anon. 1980). *R. solanacearum* was widespread in the red lateritic soils of Darwin (Heaton and Benson 1968; Aldrick 1971; Pitkethley 1981). Biotypes 3 and 4 of *R. solanacearum* were identified as being present across the Darwin region and losses in tomato from the disease could be as high as 100 % (Anon. 1980; Pitkethley 1981). The inability to produce tomatoes of suitable quality due to *R. solanacearum* meant there were few options except the adoption of grafting for local production unless a soil-less production system such as hydroponics was adopted. The cost of soil sterilization was also considered prohibitive and resistant tomato varieties were not then commercially available.

In earlier work the resistance of tomato lines to *R. solanacearum* was evaluated from 1961-1967 in Darwin (Heaton and Benson 1968). As part of the later project over 100 breeding lines were evaluated from 1979 to 1981, these were selected from over 300 lines from the Asian Vegetable Research and Development Centre in Taiwan for resistance to *R. solanacearum* that had also then been evaluated at the Laloki Horticultural Research Station near Port Moresby where biotypes 3 and 4 of *R. solanacearum* occurred (Blackburn 1982; Piggot and Blackburn 1983). Some lines had good field resistance to *R. solanacearum* in Darwin and produced large fruit in hot conditions but the fruit was not considered suitable for commercial production due the fruit being soft and of poor keeping ability.

The production of good quality tomatoes in Darwin was achieved by the grafting of commercial varieties onto wilt resistant rootstocks. The *Solanum melongena* var. wild Malay was identified as a good rootstock with resistance to both biotypes 3 and 4 of *Ralstonia solanacearum* in glasshouse inoculation studies (Anon. 1982). Thirty six indeterminate tomato varieties were evaluated as scions for grafting to *R. solanacearum* resistant stocks. Five varieties produced good yields (Blackburn and Jettner 1983; Piggot and Blackburn 1983). Grafted plants of good yielding varieties produced from 8 to14 kg per plant from May to November. In other work *Solanum torvum* (Devils fig) was also evaluated as a *Ralstonia solanacearum* resistant stock (Poffley 2003a). As *Solanum melongena* var. wild Malay and *S. torvum* are perennial plants it was reported that tomatoes grafted onto these stocks bore fruit over a longer period than non grafted tomatoes (Poffley 2003a).

Seed of *Solanum melongena* var. wild Malay was made available to the public by the Northern Territory Department of Primary Industry and Fisheries. Guides to assist with grafting were produced for the public and commercial growers to encourage the practice. Growers were also trained in grafting practices. A recent example demonstrated a cleft (also called a wedge graft locally) grafting method for *Solanum melongena* var. wild Malay stocks onto tomato scion (Traynor 2003). A commercial tomato grower grafted ~5000 plants per year and grew 'pick your own tomatoes' for the local market for 18 years up until 2009 (P. Caddy, pers. comm. Oct. 2010). All other commercial tomato production in Darwin used soil-less systems such hydroponics. Grafting was carried out using a V graft method, approximately 50-60

grafts could be produced per person per hour, this was initially carried out by the grower before contracting the grafting to an interstate nursery. The sale of cheap tomatoes from interstate lead to the ceasation of local production in 2009.

4.5.5 Case study 2: Snakebean grafting in the Northern Territory

Fusarium wilt caused by *Fusarium oxysporum* f.sp. *tracheiphilum* (Fot) was first detected on snake beans (*Vigna unguiculata* sub. sp. *sesquipedalis*) in the NT in 1999 and became a significant disease of snake beans (Conde and Arao-Arao 2001; Gosbee and Bui 2001). On farm surveys of ten properties producing snakebeans, found that eight properties had moderate to high Fot incidence levels, one had heavy levels and the tenth had low levels (B. Conde unpublished, 2009-2010).

Seventy four snake bean lines were screened and showed varying degrees of resistance to Fot. As Snake bean and Cowpea (*Vigna unguiculata* s.sp. *unguiculata*) are closely related and both are affected by Fot, various cowpea lines were also screened for resistance. Previous studies overseas have also examined the reaction of cowpeas to *Fusarium* wilt (Armstrong and Armstrong 1980; Ehlers *et al.* 2009). Four cowpea types were found to be resistant to all three strains of Fot in Darwin. The cowpea variety "Iron" was chosen as a rootstock for grafting snake beans because of its excellent resistance, strong root system and resistance to root knot nematode caused by *Meloidogyne incognita* and *M. javanica* (Conde and Arao-Arao 2003; Conde *et al.* 2010b).

A study evaluating the use of snakebean grafted to cowpea on commercial farms and an extension program for *Fusarium* wilt management in snakebean was initiated in 2008 (Conde *et al.* 2010b; Donald *et al.* 2010). The method demonstrated high levels of success in terms of protection from wilt at sites with medium and high inoculum levels (Conde *et al.* 2010b; Donald *et al.* 2010). Grafted plants at these sites with low and medium inoculum levels produced 15 and 114 % more kilograms of beans than non-grafted plants, respectively. At a high inoculum site all non-grafted plants died before production commenced, while grafted plants produced commercial yields. Grafting workshops for growers were held and printed extension materials provided to growers and the public (Conde *et al.* 2010a; Conde *et al.* 2010b; Donald *et al.* 2010).

The project demonstrated that high levels of Fot management could be obtained by the use of grafted snakebean seedlings and that associated inoculum levels were expected to be at a level that would limit yields on nine of ten farms surveyed. However, by late 2010 only a limited number of snakebean growers had adopted the practice (Donald *et al.* 2010). Approximately 50 growers produce snakebeans in the Darwin region over the tropical dry season (S. Smith pers.comm.), with a large percentage of the produce shipped to southern Australian markets where attractive out of season prices are obtained. Thus the incentive to ensure high production levels is considered high.

A number of facilities and conditions were identified as necessary for the successful propagation of grafted snakebean seedlings (Donald *et al.* 2010). These included: seed trays for production of stock and scion seedlings; disease free potting media; after-graft nursery facilities including a high humidity tent; supports for seed trays with scion seedlings to be kept off the ground to prevent soilborne infection; the use of watering systems that prevented soil splash. Snakebean production in the NT has been based on direct seeding methods. For most growers, the propagation of seedlings, equipment and resources required would be novel requirements. These requirements are also additional to the development of skills in the areas of seedling propagation, plant hygiene and irrigation practices, grafting techniques and graft after-care husbandry.

Although snakebean grafting requires additional resources, this investment was more than compensated for by the production of grafted plants where Fusarium wilt caused losses. A comparison of estimated production costs including grafting and slight (10 % yield loss), moderate (50 % yield loss) or severe (100 % yield loss) losses in yield to snakebean wilt was made (Appendix 4.2). Grafting cost an estimated additional $1025 per 1000 plants, grafting gave an estimated increase in net returns after including grafting costs of $887, $8,537 and $18,100 for slight, moderate and severe losses based on an average yield per plant of 4.5 kg. Higher net returns of $3,012, $19,162, and $39,350 for the slight, moderate and severe levels of yield loss were estimated for grafted plants yielding 9.5 kg per plant.

Perceptions of bean productivity from grafted seedlings may affect uptake by growers. Some growers have claimed that uninfected seedlings yield more than grafted plants, however, data from two trials at a commercial sites using the same plant density did not support this (data not presented). However, growers typically sow two snakebean seeds together and the seedlings are not thinned to single seedling if both emerge. Where production levels from two non-grafted seedlings are being compared to a single grafted seedling, the production for the single grafted seedling is expected to be lower. Double density plantings of grafted seedlings could be planted for comparison with the two seedling plantings and the results used to support extension activities.

The use of grafted snakebean seedlings is still a relatively new practice in Darwin. Two prominent growers have adopted the practice with one of these growers organising a local agrichemical company to supply stocks of the cowpea variety for use as a rootstock. With time and contact between growers a greater uptake of grafting practices may be

obtained. This case study indicates that industries that are not experienced with propagation or the use of transplanted seedlings may have greater barriers to adoption than transplant based industries.

4.5.6 Factors affecting uptake of grafting

The two cases studies from the Northern Territory provided some ability to propose factors associated with the uptake and commercial use of grafting for soilborne disease management. Two factors in particular are noteworthy, the prevalence of the pathogen of concern and propagation systems used by growers.

In the case of *Ralstonia solanacearum* in tomatoes, the pathogen was regarded as widespread and possibly indigenous to the Darwin region (Heaton and Benson 1968; Aldrick 1971; Pitkethley 1981). The growing of tomatoes was unsuccessful after a short period due to both the prevalence and susceptibility due to a lack of resistance by cultivars at the time to biotypes 3 and 4. Thus grafting was apparently the only method of soil grown tomato production available unless soil fumigants were used. In contrast *Fusarium oxysporum* f.sp. *tracheiphilum* (Fot) was first found on snake beans in 1999, this was approximately 10 years after commercial snakebean production had been established in the region. Growers of Asian vegetables in Darwin are reported to make use of new land each year to avoid root knot nematode problems (Conde *et al.* 2005). Up until relatively recently growers of snakebeans had been able to use land where snakebeans were not previously grown, although a recent survey indicated that existing farms have, on average, had inoculum levels associated with moderate to high Fot incidence levels (B. Conde unpublished, 2009-2010). Thus growers of snakebeans may have until recently considered that they may avoid the *Fusarium* wilt of snakebean by moving production. By contrast, losses of tomatoes to bacterial wilt had occurred since at least 1961 and, due to the prevalence of *Ralstonia solanacearum,* shifting production would not be considered a viable management option. These differences in inoculum distribution between the two pathogens and the period of time since the disease has been a major porblem may affect the immediacy of uptake of grafting to manage soilborne pathogens.

Tomatoes are a crop where the use of transplants is the norm for both field and glasshouse production in Australia. By contrast snakebeans in the Northern Territory are usually direct seeded. Thus propagation materials (trays, seed mix, seedling houses etc.) or the purchasing of seedlings are standard procedures in the production of tomatoes but not for snakebean production. Propagation materials for the production of rootstocks and scions for use in grafting and after-care facilities are required for grafted seedlings. For growers who do not normally propagate or purchase seedlings, the investment required for grafted seedlings may appear unacceptably large.

The differing propagation experience and investment between snakebean and tomato growers and differing historical prevalence of the respective target pathogens appear to provide some initial explanations for the differing scale of uptake of grafting for disease management between the two crops in the Darwin region.

4.5.7 Potential for grafting in other Australian vegetable crops

The overseas literature contained a useful number of reports of the use of grafting to manage soilborne pathogens and this strategy has been evaluated for four soilborne diseases in Australia. To the authors knowledge the commercial use of grafting to manage soilborne pathogens in Australia remains limited.

For the wider use of grafting in Australia, the most immediate strategy would be to evaluate and implement successful grafting stocks and methods for problem pathogens in Australian vegetables where these have already been developed internationally. A literature search indicated that, on the basis of overseas studies, grafting of resistant rootstocks may assist with the management of four pathogens in the target genera that have been recorded on three Australian vegetable crops (tomato, melon and watermelon) (Table 4.16). Two other crops, snakebeans and cucumber have had grafting evaluated in Australia. Eleven other soilborne pathogens recorded in six Australian crops from a range of genera were also recorded to be managed by grafting practices (Table 4.17).

Grafting may also be of use in the management of soilborne pathogens in Australia that have not had grafting techniques or resistant rootstocks developed or evaluated overseas. Progress in this area would require fundamental research in Australia. This approach may have merit and should not be discounted, however, this review has focused primarily on crop-pathogen-grafting systems that have already been developed nationally or internationally.

Management potential for fungal soilborne pathogens in Australian crops by grafting

A number of pathogens in the group of four targeted soilborne pathogen genera (*Pythium, Fusarium, Sclerotinia* and *Rhizoctonia*) were reported to be managed through the use of grafted resistant rootstocks, these included specific species and in some cases *forma specialis* that occur in Australia (Table 4.16). This also included some pathogens such as *Fusarium oxysporum* f.sp. *lycopersici* (race 3) which was regarded as still spreading in distribution in 2003 and that

the disease incidence and severity would be likely to increase in the future (Stirling and Ashley 2003). There were no records for the use of grafting for management of disease in the genera *Rhizoctonia* and *Sclerotinia*, which were identified by Donald *et al.* (2010) as important in Australia. As pathogens in this genera generally have a wide host range and infect by killing tissue as colonising there may be limited opportunities to identify resistant rootstocks that are also closely related to the susceptible scion and so would be suitable for use as rootstocks.

Table 4.16. Reports of grafting to control four Genera (*Sclerotinia*, *Rhizoctonia*, *Pythium* and *Fusarium*) of important soilborne pathogens in Australian vegetable crops and reports of grafting methods to control this pathogen in the same crop.

Crop	Pathogen	Citation	Stock	Citation
Beans (Snake)	*Fusarium oxysporum* f.sp. tracheiphilum	(Gosbee and Bui 2001)	*Vigna unguiculata*	(Conde *et al.* 2010b)
Capsicum				
Eggplant				
Cucumber	*Pythium aphanidermatum*	(Tesoriero 2009)	Cucumber	(Tesoriero 2009)
	Fusarium oxysporum f.sp. radicis-cucumerinum	(Tesoriero and Bertus 2004)	Cucumber	(Pavlou *et al.* 2002)
	Fusarium oxysporum f.sp. cucumerinum	(Wicks *et al.* 1978; Persley *et al.* 1989; Tesoriero 2009)	Cucumber	(Lee and Oda 2003; Tesoriero 2009)
Tomato	*Fusarium oxysporum* f.sp. *lycopersici* (races 1, 2, 3)	(Grattidge and Obrien 1982; Ramsey *et al.* 1992; Stirling and Ashley 2003)	Tomato Multiple stock species listed by Lee and Oda (2003)	(Lee and Oda 2003; Miguel 2005b; Rivard and Louws 2008)
	Sclerotium rolfsii	(Stirling and Ashley 2003)	Tomato	(Rivard *et al.* 2010)
Melons	*Fusarium oxysporum* f. sp *melonis*	(Persley *et al.* 1989; Anon. 2010)	Melon Benincasa Cucumis species Cucurbita species Lagenaria	(Nisini *et al.* 2002; Crino *et al.* 2007)
Watermelon	*Fusarium oxysporum* f. sp *niveum*	(Persley *et al.* 1989; Anon. 2010)	Watermelon, Lagenaria, and Cucurbita	(Miguel *et al.* 2004; Taylor *et al.* 2008; Keinath *et al.*2010)

A number of other soilborne pathogens recorded in Australia were also recorded as being managed by the grafting of resistant rootstocks in studies, these are listed in Table 4.17.

Table 4.17. Records of soilborne fungal pathogens and non-fungal pathogens in Australian vegetable crops and reports of grafting methods to manage this pathogen in the same crop, exclusive of four Genera (*Sclerotinia*, *Rhizoctonia*, *Pythium* and *Fusarium*).

Crop	Pathogen	Citation	Stock	Citation
Capsicum	*Phytophthora nicotianae*	(Anon. 2010)	Capsicum 'Brutus'	(M'Hamdi *et al.* 2010)
	Meloidogyne incognita	(Vawdrey and Stirling 1996)	Capsicums	(Kokalis-Burelle *et al.* 2009)
	Ralstonia solanacearum [Pseudomonas solanacearum]	(Pitkethley 1981; Anon. 2010)	Capsicums	(Wu *et al.* 2008a)
Eggplant	*Verticillium dahliae*	(Anon. 2010)	Tomato, *Solanum torvum, S. sisymbriifolium*	(Arvanitoyannis *et al.* 2005; Bletsos 2006; Liu *et al.* 2009)
	Meloidogyne incognita	(Vawdrey and Stirling 1996)	Tomato*	(Ioannou 2001)
	Ralstonia solanacearum	(Anon. 2010)	Tomato	(Gordon 1994)
Cucumber	*Verticillium dahliae*	(Anon. 2010)	Two cucumber and one watermelon	(Paplomatas *et al.* 2002)
Tomato	*Meloidogyne* spp.	(Vawdrey and Stirling 1996; Stirling and Ashley 2003)	Tomato	(Rivard *et al.* 2010)
	Ralstonia solanacearum	(Heaton and Benson 1968; Pitkethley 1981)	Eggplant *Solanum torvum* Tomato	(Poffley 2003a; Cardoso *et al.* 2006; Palada and Wu 2007; Rivard and Louws 2008)
	Sclerotium rolfsii	(Stirling and Ashley 2003)	Tomato	(Rivard *et al.* 2010)
	Verticillium dahliae	(Stirling and Ashley 2003)	3 tomato stocks	(Paplomatas *et al.* 2002)
Melons				
Watermelon	*Meloidogyne incognita*	(Vawdrey and Stirling 1996)	Watermelon	(Huitron *et al.* 2007; Thies *et al.* 2010)

*Management in winter only

Pathogens addressed by grafting studies: Disease records for Australia indicated that grafting addressed some important diseases for the selected crops, but a number of diseases did not appear to have been addressed for management potential in grafting studies, or included diseases reported to be of minor importance. Industry knowledge of the current status of a number of pathogens including which races are now present may also be required as pathogen databases and reports may not adequately reflect the current status of pathogens of economic concern for some crops. This section identifies the level of pathogen coverage by grafting for specific crops in Australia.

Beans: There was no indication that soilborne *Pythium* or *Sclerotinia* had been managed through a grafting strategy in *Phaseolous* species. Although, experimental grafting work on *Phaseolous* species has found that a vigorous rootstock of *Phaseolous vulgaris* could increase yield from 66-91% (Gurusamy *et al.* 2010). Experimental studies on *Phaseolous vulgaris* have also indicated that grafting (a *Fusarium solani* f.sp. *phaseoli* resistant *P. vulgaris* kidney bean cv. FR266) to a susceptible *P. vulgaris* snap bean cultivar was successful to protect the scion from infection by *Fusarium solani* (Cichy *et al.* 2007). *Fusarium solani* f.sp. *phaseoli* is listed as part of a complex with *Aphanomyces sp.* and *Pythium sp.* that can cause serious losses in French beans in Australia (Persley *et al.* 1989). *Sclerotinia sclerotiorum* are common pathogens of field beans in Australia (Merriman *et al.* 1979; Anon. 2010). However, knowledge of the prevalence and severity of these pathogens in tunnel or glasshouse production of climbing or runner beans where a single plant may be picked for an extended period appears lacking. One report, however, indicated that *Pythium* was a significant problem in Northern Territory snakebean production associated with water logged ground (Donald *et al.* 2010).

Capsicum: *Pythium myriotylum* or *P. aphanidermatum* are serious pathogens in Capsicum (Vawdrey and Stirling 1996). The literature on capsicum grafting did not indicate a prior record for the management of pathogens noted as important in Australian capsicum production. Although, capsicum were noted as being more susceptible to

Meloidogyne incognita than *M. javanica* in Queensland (Vawdrey and Stirling 1996) and tolerant *M. incognita* capsicum cultivars have been identified overseas (Kokalis-Burelle *et al.* 2009). A *Phytophthora sp.* was recorded as a root rot disease in greenhouse capsicum and chilli (Tesoriero and Bertus 2004) and *Phytophthora nicotianae* was recorded on capsicum in New South Wales (Anon. 2010). An intraspecific stock was used to manage *Phytophthora nicotianae* in capsicum (M'Hamdi *et al.* 2010). The relevance of the use of a rootstock would depend on the importance of the disease.

Cucumber: *Fusarium oxysporum* f.sp. *radicis-cucumerinum* in association with a *Pythium* which was possibly *Pythium aphanidermatum* caused severe disease in cucumber, with crop losses as high as 30 % (Tesoriero and Bertus 2004). In later work the taxonomic discrimination between *Fusarium oxysporum f.sp. radicis-cucumerinum* and *F. oxysporum* f.sp. *cucumerinum* was questioned (Tesoriero 2009). But *F. oxysporum* f.sp. *radicis-cucumerinum* was reported to be managed with grafting in Europe (Pavlou *et al.* 2002). Previously *F. oxysporum* f.sp. *cucumerinum* had been of concern in Australian cucumber production (Wicks *et al.* 1978). A study in 2009 identified that two important soilborne pathogens of cucumber were *Pythium aphanidermatum* and *Fusarium oxysporum* f.sp. *cucumerinum* causing a disease complex and that there was management potential of this complex by intraspecific grafting in Australia (Tesoriero 2009). To manage *F. oxysporum* f.sp. *cucumerinum* in Australia, stocks resistant to race 3 are required (L. Tesoriero, pers. comm. Nov. 2010). Further work to develop the commercial use of this strategy appears to be required.

Eggplant: No pathogens in the group of four target genera such as *Pythium vexans* were reported to be managed in eggplant by grafting. But a number of other pathogens were reported to be managed by grafting. Some control by grafting of a nematode species reported as *Meloidogyne incognita,* important in Queensland, was also reported however, eggplant is also affected by *Meloidogyne javanica* (Vawdrey and Stirling 1996; Ioannou 2001).

Tomato: A large number of diseases are reported for tomato in Australia (Anon. 2010). There are multiple reports of the use of grafting to manage an important disease caused by *Fusarium oxysporum f.sp. lycopersici* (Miguel 2005b; Rivard and Louws 2008). Most of these reports concern races 1 and 2, but as race 3 is also present, stocks resistant to this race would be required (Stirling and Ashley 2003). The ability to select stocks that are also resistant or tolerant to *Meloidogyne incognita* and *Meloidogyne javanica* may be especially important for providing multiple pathogen management benefits from grafting.

Melons and Watermelon: There were multiple reports for the management of both *Fusarium oxysporum* f.sp. *melonis* and f.sp. *niveum* by grafting. Detailed knowledge of the occurrence of the particular races in production areas in Australia for each forma specialis will be required for the selection of suitable stocks to assist with management. Similar to tomato, there also appears to be an opportunity to select stocks with tolerance to *Meloidogyne incognita* as *M. incognita* causes losses in watermelon in Queensland (Vawdrey and Stirling 1996).

4.5.8 Determining feasibility

This section addresses the question of feasibility of grafting as a practice in terms of both practical and economic requirements.

Cost:benefit balance

Although there are numerous examples of the successful management of soilborne pathogens by grafting on to resistant rootstocks, the production of grafted seedlings clearly requires additional skilled labour, time, space and materials (Oda 1995). For the practice to be a feasible commercial method of pathogen management these additional costs of production need to be covered by the potential costs in lost production due to disease losses or secondary costs. This section identifies the various components contributing to grafting costs and details findings from economic analyses of grafting for disease management. The choice of crop and production system used also affects the cost structure. For example, in Europe for glass house production of long season tomatoes, production of grafted transplants cost 8 % of the total growing cost compared to 4.7 % for non-grafted (Miguel 2005b). Long season tomato crops can be picked over a number of months and the overall cost structure of glasshouse production will be much higher than field production. In contrast to a number of field grown vegetable crops such as capsicum in Queensland, the predominant practice is for the production of single harvest crops (E. Jovicich pers comm.). The proportional cost of grafted transplants to total production costs may be expected to be higher in this later system.

Cost of propagation: Grafting requires additional propagation costs as two lots of seed, two lots of seedlings, trays, potting mix and facilities for seedlings are required. Suitable space for this additional production of both stock and scion is required so that they can both be produced at suitable compatible stages of development for grafting.

Some rootstocks are from species or crosses that are not common agricultural crops and may require specialised knowledge to source or import seed. However, a number of studies showed that the use of intraspecific stocks (of the

same species) can provide effective protection against a number of important pathogens (Cohen *et al.* 2002; Rivard and Louws 2008). Tesoriero (2009) used this method successfully for cucumber in Australia. In addition to not having to source or import different species the use of intraspecific combinations may be useful to restrict the amount of trial work required to determine rootstock:scion incompatibility as was experienced with a number of interspecifc (closely related species) rootstock:scion combinations (Yetisir *et al.* 2003; Davis *et al.* 2008a).

Cost of grafting and after-care: The speed of grafting operations is one means of comparing the costs of different systems. In a comparison of three methods the Tongue or Approach graft was slower (71.4 min/100 plants) than two other methods the Hole Insertion and Pin techniques (60.3 and 46.0 min/100 plants, respectively) (Davis *et al.* 2008b). Automated grafting methods have been developed to improve efficiencies. Automated grafting can be used to produce large numbers of 400-1200 grafts per hour (Lee and Oda 2003). Another automated robot grafter produced 750 grafts per hour (Kubota *et al.* 2008). Unfortunately, the economic studies that include the purchase cost of this machinery on the costs of production appear not to have been carried out. Lee (1994) estimated that 7 to 10 days of labour were required for the careful management of seedlings in grafting after-care, but also noted that this labour requirement could be reduced by the use of specially designed conditioning chambers.

Purchase cost of grafted seedlings: A number of studies provide a total cost for the purchase of grafted seedling produced by commercial grafting companies. Although, not clearly stated it is assumed that this cost covers all additional costs associated with grafting including materials, propagation, grafting and after-care (Davis *et al.* 2008b; Taylor *et al.* 2008). In some areas self-grafting by growers has been largely replaced by cooperative and commercial operations (Lee 1994).

Grafted seedlings cost more than non-grafted. Examples include: Cucurbit seedling costs can be four times those of non-grafted seedlings in Japan and the United States (Davis *et al.* 2008b); grafted watermelon transplants in the United States purchased from a commercial producer cost 2.6 times those of non-grafted seedlings (Taylor *et al.* 2008); grafted melon seedlings cost US $ 1221 per ha for two crop cycles in comparison to $ 542 for non-grafted in Central America (Amadio 2005).

The cost of grafted seedlings was used as a basis to compare the economics of the use of grafted seedlings for *Fusarium* wilt management in watermelon production in the USA (Taylor *et al.* 2008). The grafted watermelon transplants used in the study were purchased from a commercial producer and cost $ US 0.75 compared to non-grafted at $ US 0.28, at 3700 plants/ha the additional cost was US $ 1743 ha. It was calculated that to pay for these costs of grafted seedlings an additional US $ 0.06 per kg of watermelon would be required to provide the same net return of ungrafted watermelon from yield of 40,000 kg/ha.

Taylor *et al*. (2008) identified that the level of risk was an important factor affecting the cost:benefit outcome. For example, if a farmer had a good yielding field (50,000 kg/ha) that had continued to provide stable yields and no history of *Fusarium* wilt problems, then investment in grafted transplants should not be considered as the risk of losses due to *Fusarium* wilt will be considered low. In contrast a farmer with a field that used to yield 50,000 kg/ha who had since experienced yield decline to 25,000 kg/ha might expect that the use of non-grafted transplants would lose $ US 198 ha but a yield of 50,000 kg/ha from grafted transplants would give a $ 560 ha profit.

The study concluded that as *Fusarium* wilt occurs late in the production season most of the costs of watermelon production have already been spent, therefore, decisions over use of the more expensive transplants need to be based the level of risk of particular fields, costs and probable revenue.

Basis for cost comparisons of grafting

Establishing the basis for cost comparisons of grafted seedlings can be complex due to the potential inclusion of a number of additional factors including: agrichemicals; plant density, land availability, inoculum and disease risk levels and the inclusion of additional benefits to those of disease management in costings.

Agrichemicals: If the costs of reductions or elimination of fungicides or soil fumigants are included comparative costings, the costing of grafted seedlings can be more favourable (Davis *et al.* 2008b). Some examples are: If the cost of a methyl bromide treatment was included, grafted production (Francs 9/m^2) was cheaper than non-grafted production (Francs 9.8/m^2) of greenhouse tomatoes in France (Miguel 2005b). In Central America the standard practice of soil fumigation with methyl bromide, plastic film and nongrafted seedlings cost US $ 0.06 /m^2 which was the same cost as no soil treatment and the grafted seedling (Amadio 2005). However, for regions where methyl bromide had been phased out it has been argued that the cost should be not be included in comparisons as methyl bromide is no longer available as a management option (Taylor *et al.* 2008). Methyl bromide fumigation costs were estimated at ~ $2500 ha for tomato for production at 12000 plants per ha (Stirling and Ashley 2003). However, comparison with previous practices as one of multiple scenarios may be useful for evaluating the long term economics of differing management systems.

Plant density: In some grafted production systems, for example watermelon in Israel, grafted plants are planted at a lower density than non-grafted due to the higher production per plant (Cohen *et al.* 2007). The economics of the use of lower densities of grafted plants appear not to have been examined.

Land availability: Where broadacre growers can gain access to new uninfected land at the same cost as their existing land and production costs at the new site remain the same, the cost of managing a soilborne pathogen can be avoided. But where new land costs more, production is limited or production costs are increased due to greater transport costs for example, then the strategy of shifting production is not fully successful as a means of avoiding the costs the soilborne pathogen. Land availability will differ across Australia and may also differ between crops in terms of land suitability. Grafting may be more economically viable for broadacre production where land availability is limited or it increases production costs. Economic analyses are required in this area. In contrast for production systems such as glasshouse and tunnel houses the high establishment costs at a site would probably make shifting sites a less economically viable alternative for soilborne pathogen avoidance than for broadacre production, however, again economic analyses are required in this area.

Importance of identifying disease risk: A major conclusion from an analyses of the economics of watermelon grafting in the USA was the greater relative return where disease levels were limiting production (Taylor *et al.* 2008). Therefore, an accurate knowledge of the inoculum levels of the pathogens of concern at a site are required in addition to reliable disease risk predictions based on inoculum and management factors. The evaluation of soilborne inoculum concentrations in cereal fields for cereal diseases in Australia and associated disease risk levels on a commercial basis has provided a useful means to selecting annual crops for sites (Ophel-Keller *et al.* 2008). This service also includes testing soil concentrations for *Fusarium pseudograminearum* and *F. culmorum*. In other work initial assays have been developed for *Meloidogyne* spp. and *Fusarium oxysporum* f. sp *lycopersici* detection and disease risk assessments for fields to be planted to tomatoes (Stirling *et al.* 2004b). Substantial progress has been made identifying sequences for differentiating different formae specialis of *F. oxysporum* (O'Donnell *et al.* 2009), therefore, extension of the existing technology to include additional pathogens including multiple formae specialis of *F. oxysporum* appears feasible. In addition, as the returns from horticultural crops are generally higher per unit area of land than cereal crops the testing of soils from horticultural sites for concentrations of key soilborne pathogens may represent a relatively small investment relative to the costs of production or losses in production should disease levels be significant. The development of inoculum quantification and associated disease risk levels for important horticultural crops would provide a useful tool for assisting growers to determine when grafted transplants on resistant stock may be required at a particular site, or if these additional costs are not necessary due to low inoculum pressure and low disease risk levels.

Other disease management or additional benefits: A number of studies cited improved management of non-soilborne pathogens (Lee and Oda 2003), yields of grafted plants exceeding previous non-grafted production levels even in the absence of a limiting pathogen (Taylor *et al.* 2006), more stable yields over time (Miguel *et al.* 2004); improved quality attributes (Taylor *et al.* 2006), or the ability to produce crops for market over a wider harvest period such as early or late season for which greater prices may be obtained (Burleigh *et al.* 2005; Palada and Wu 2005; Palada and Wu 2007). In these cases important financial benefits have been obtained from the use of grafted transplants additional to that of soilborne pathogen management. These additional benefits clearly should be included in any cost:benefit analysis.

Due to the potential importance of these additional factors on the economic viability for the use of grafting to manage soilborne pathogens, it would appear that future strategies for the use of resistant stocks should target crops and rootstocks where some of the above benefits can potentially be obtained.

Potential limitations

Current limitations to the use of grafting for important soilborne pathogen management in vegetable crops in Australia are: accurate information on which rootstocks can successfully be used to manage diseases in an Australian environment especially with the occurrence of high soil temperatures; the availability of resistant stocks; the ability/availability of commercial companies able to provide grafted seedlings of a suitable quality and price for use in large scale production; an absence of information on the economics of using grafted seedlings principally for soilborne pathogen management.

4.5.9 Conclusions

There was clear evidence from a number of overseas studies that there was potential for the use of resistant rootstocks to manage specific soilborne pathogens in Australian vegetable production. The ability of this practice to be used in Australia was also demonstrated by a number of studies and the use of grafting to manage *Fusarium* wilt in snakebean in the Northern Territory (see 4.3.2) and bacterial wilt in eggplant and tomato in Queensland and the Northern Territory, respectively. For a number of overseas studies high soil temperatures were recorded as negatively affecting the level of management, this area may require specific examination when evaluating the potential of grafting under Australian conditions.

The feasibility of the commercial use of these practices was a more complex issue to make clear conclusions on, especially as the cost of grafting is relative to the overall cost of the production system used. Grafting appears to be more feasible for high production level and long season (multiple pick) systems such as glasshouse or protected cover systems, although field grown crops that are trellised for multiple pick production may also be suitable. Due to the importance of the cost of propagating and grafting seedlings, economic analyses will be required to determine commercial feasibility. A number of specific economic analyses were recommended as necessary to determine the commercial feasibility of grafting. Due to the additional cost grafting adds to a crop, rootstocks that also offer additional production benefits (improved quality or volume, longer production, tolerance to stress) should be selected so that multiple (improved disease and production) benefits may be obtained from grafting. If multiple benefits can be gained from the use of grafting, then the economic feasibility of this practice will be improved.

4.6 Acknowledgements

Barry Condè and Mark Traynor conducted field trials, workshops, field days and race differential studies. These authors thank the farmers, Van Son Chau, An Tran, Manh Van Nguyen and Paul Dinh who cooperated with the on-farm trials on their farms and also assisted by recording the yields from the seedling and grafted rows. In addition the following are acknowledged for their assistance:
Mark Hearnden, Dept of Resources for his biometrical services.
Caroline Donald, Department of Primary Industries Victoria for her guidance and encouragement.
Ian Porter, Department of Primary Industries Victoria for encouraging us to be a part of this national project.
Dale Cumberland and Lois Ulyatt Plant Pathology Branch, Dept of Resources for their technical assistance and Lucy Tran-Nguyen for helpful discussions and encouragement.
Rachel Meldrum for her 2006 study of the diversity of Fot strains on three farms in the Darwin area.
Lynne Cooke, Vincent Nott, Kathy Roe and Kate Verus of the Berrimah Farm Library for literature reviews and assistance obtaining references.
Sal Norton, Merv. Rettke and Peter Lawrence, Biloela for seed and for importing seed lines from overseas.
Arthur Cameron for seed of NT lines.
Professor Yi-Sheng Lin, Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan, R.O.C. for unpublished results.
Jeff Ehlers Plant Breeder, UC-Riverside for advice, for providing the PN Patel and Ehlers race differential table, and for providing their cowpea race differentials, and Marti Pottorff, PhD student, UC-Riverside for providing a list of lines screened against races 1 to 4 and for discussions on race differentials.
We thank Stuart Smith for co-ordinating the end of season field event November 25th 2010, and Mark Traynor, Sean Bithell, Megan Connelly, Les Huth, Mark Hoult, Peter Bergin, Geoff Walduck, Brian Thistleton, Graham Schultz, Ben Beumer, Charmayne Craven, Matthew Henger, Andrea Ruske, Anna Grosvenor, Joan Barton, Chelsea Moore, Jason De Araujo, Christine Long, Peter Stephens, Arthur Cameron, and Bruce Sawyer for their inputs into the November 25th 2010 field event.

Dr. Lucy Tran-Nguyen conducted molecular characterisation of Fot. This author acknowledges Barry Condè, Dale Cumberland, Lois Ulyatt and Joanne Tilbrook, Plant Pathology Section, Department of Resources for their contributions to this study. Dr. Philip Roberts (University of California, Riverside) for the *Fot* races 3 and 4 DNA.

Sean Bithell reviewed the potential of grafting to improve soilborne pathogen management in high value vegetable production in Australia. This author acknowledges Department of Resources Berrimah Farm Library staff for identifying and sourcing literature.

4.7 References

Agrios GN (2005) Plant Pathology, 5th Edition. Elsevier Academic Press, pp 922.

Aldrick SJ (1971) Bacterial wilt (*Pseudomonas solanacearum*) of *Stylosanthes humilis* in the Northern Territory. *Tropical Grasslands* **5**, 23-26.

Amadio A (2005) Alternatives to methyl bromide adopted for cucurbit production in projects funded by the Montreal Protocol. In 'Proceedings of the VIth International Symposium on Chemical and Non-Chemical Soil and Substrate Disinfestation'. (Ed. A Vanachter) pp. 71-74. (International Society Horticultural Science: Leuven 1).

Anderson JB, Stasovski E (1992) Molecular phylogeny of Northern Hemisphere species of *Armillaria. Mycologia* **84,** 505-516.

Annamalai P, Ishii H, Lalithakumari D, Revathi R (1995) Polymerase chain reaction and its application in fungal disease diagnosis. *Journal of Plant Disease Protection* **102,** 91-104.

Anon (1980) Plant Industry Branch Annual report 1979-80. Department of Primary Production, Northern Territory Government. 6-7, Darwin.

Anon (1982) Annual Report 1981-82. Plant Industry Branch, Department of Primary Production, Northern Territory Government. 37-38, Darwin.

Anon (2010) Australia Plant Pest Database Accessed October 2010
http://www.planthealthaustralia.com.au/go/phau/capacity-and-capability/information-support-systems/appd

Appel DJ, Gordon TR (1995) Intraspecific variation within populations of Fusarium oxysporum based on RFLP analysis of the intergenic spacer region of the rDNA. *Experimental Mycology* **19,** 120-128.

Armstrong GM, Armstrong JK (1950) Biological races of the Fusarium causing wilt of cowpeas and soybeans. *Phytopathology* **40,** 181-193.

Armstrong GM, Armstrong JK (1965) Wilt of chrysanthemum caused by Race 1 of the cowpea Fusarium. *Plant Diseases Rep*. **49,** 673-676.

Armstrong GM, Armstrong JK (1980) Cowpea wilt *Fusarium oxysporum* f. sp. *tracheiphilum* race 1 from Nigeria. *Plant Disease* **64(10),** 954-955.

Arvanitoyannis IS, Khah EM, Christakou EC, Bletsos FA (2005) Effect of grafting and modified atmosphere packaging on eggplant quality parameters during storage. *International Journal of Food Science and Technology* **40**, 311-322.

Assunção IP, Michereff SJ, Mizubuti ESG, Brommonschenkel SH (2003) Influência da Intensidade da Murcha-de-Fusário no Rendimento do Caupi. *Fitopatologia Brasileira* **28**, 615-619.

Bentley S, Pegg KG, Dale JL (1995) Genetic variation among a world-wide collection of isolates of *Fusarium oxysporum* f. sp. *cubense* analysed by RAPD-PCR fingerprinting. *Mycological Research* **99,** 1378-1384.

Bentley S, Pegg KG, Moore NY, Davis RD, Buddenhagen IW (1998) Genetic variation among vegetative compatibility groups of *Fusarium oxysporum* f. sp. *cubense* analysed by DNA fingerprinting. *Phytopathology* **88,** 1283-1293.

Blackburn KJ (1982) Bacterial wilt resistance project. Summary of work Sepember 1979-1981. Plant Industry Branch, Department of Primary Production, Northern Territory Government. 12, Darwin.

Blackburn KJ, Jettner CJ (1983) Tomato research programme: Grafted tomato varietial assessments. Plant Industry Branch, Department of Primary Production, Northern Territory Government. 2, Darwin.

Bletsos FA (2006) Grafting and calcium cyanamide as alternatives to methyl bromide for greenhouse eggplant production. *Scientia Horticulturae* **107**, 325-331.

Bletsos FA, Moustafa AM, Thanassoulopoulos CC (2002) Replacement of methyl bromide application by alternative methods in vegetables under cover. *Proceedings of the Second Balkan Symposium on Vegetables and Potatoes*, 451-456.

Booth C (1971) The genus Fusarium. Commonwealth Mycological Institute, Kew, Surrey, England. pp237.

Burleigh JR, Black LL, Mateo LG, Cacho D, Aganon CP, Boncato T, Arida I, Ulrichs A, Ledesma DR (2005) Performance of Grafted Tomato in Central Luzon, Philippines: A Case Study on the Introduction of a New Technology Among Resource-limited Farmers. *Crop Management*, 9.

Cabi Compendium
http://www.cabi.org/cpc/default.aspx?site=161&page=868

Cai G, Rosewich Gale L, Schneider RW, Kistler HC, Davis RM, Elias KS, Miyao EM (2003) Origin of race 3 of *Fusarium oxysporum* f. sp. *lycopersici* at a single site in California. *Phytopathology* **93,** 1014-1022.

Cardoso SC, Fermino Soares AC, Santos Brito AD, De Carvalho LA, Da Silva Ledo CA (2006) Potential of Hawaii 7996 hybrid as rootstock for tomato cultivars. *Bragantia* **65**, 89-96.

Chung H-D, Ryu T-S, Choi Y-J (2003) Selection of salt-tolerant bottle gourd (*Lagenaria siceraria*) rootstock for watermelon graft. *Journal of the Korean Society for Horticultural Science* **44**, 588-594.

Cichy KA, Snapp SS, Kirk WW (2007) Fusarium root rot incidence and root system architecture in grafted common bean lines. *Plant and Soil* **300**, 233-244.

Cohen R, Burger Y, Edelstein M (2004) Grafted Melons: The Phytopathological Aspect. *Hortscience* **39**, 873-873.

Cohen R, Burger Y, Horev C, Koren A, Edelstein M (2007) Introducing grafted cucurbits to modern agriculture - The Israeli experience. *Plant Disease* **91**, 916-923.

Cohen R, Horev C, Burger Y, Shriber S, Hershenhorn J, Katan J, Edelstein M (2002) Horticultural and pathological aspects of Fusarium wilt management using grafted melons. *Hortscience* **37**, 1069-1073.

Conde B, Arao-Arao I (2001) Management Systems for Diseases of Asian Vegetables - Fusarium Wilt of Snake Beans. Technical Annual Report 2000-2001, Primary Industries, Northern Territory Government. 198-199.

Conde B, Arao-Arao I (2003) Management System for Fusarium Wilt of Snake Beans. Technical Annual Report 2002-2003, Primary Industries, Northern Territory Government. 232-234.

Condé BD, Arao-Arao I. Pitkethley RN (2003) Screening snake bean and cowpea varieties and lines in a glasshouse for resistance to Fusarium wilt of snake beans in Darwin, northern Australia. Proceedings of the 6th MAPPS Conference, Kuala Lumpur, Malaysia.

Conde, B, Arao-Arao, I, Pitkethley, R , Owens, G and Traynor, M (2010a). Grafting snake beans to control Fusarium wilt. Agnote. (I61): 3 pp. Department of Primary Industries and Fisheries, Northern Territory of Australia. Darwin, Australia.

Conde B, Pikethley R, Arao-Arao I (2005) Root knot nematode disease. Information sheet No. 12. 2.

Conde B, Traynor M, Cumberland D, Hearnden M, Tran-Nguyen L, Tillbrook J, Ulyatt L (2010b) Control of Snake bean Fusarium wilt in the NT. Primary Industries Annual Research Report 2009-2010. Technical Bulletin 335. Plant Industries, Department of Resources, Northern Territory Government. 131-136.

Correll JC, Klittich CJR, Leslie JF (1987) Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* 77, 1640-1646.

Correll JC, Puhalla JE, Schneider RW (1986) Identification of *Fusarium oxysporum* f.sp *apii* on the basis of colony size, virulence and vegetative compatibility. *Phytopathology* **76**, 396-400.

Crino P, Lo Bianco C, Rouphael Y, Colla G, Saccardo F, Paratore A (2007) Evaluation of rootstock resistance to fusarium wilt and gummy stem blight and effect on yield and quality of a grafted 'Inodorus' melon. *Hortscience* **42**, 521-525.

Curuk S, Dasgan HY, Mansuroglu S, Kurt S, Mazmanoglu M, Antakli O, Tarla G (2009) Grafted eggplant yield, quality and growth in infested soil with *Verticillium dahliae* and *Meloidogyne incognita*. *Pesquisa Agropecuaria Brasileira* **44**, 1673-1681.

Davis AR, Perkins-Veazie P, Hassell R, Levi A, King SR, Zhang XP (2008a) Grafting effects on vegetable quality. *Hortscience* **43**, 1670-1672.

Davis AR, Perkins-Veazie P, Sakata Y, Lopez-Galarza S, Maroto JV, Lee SG, Huh YC, Sun ZY, Miguel A, King SR, Cohen R, Lee JM (2008b) Cucurbit grafting. *Critical Reviews in Plant Sciences* **27**, 50-74.

Donald C, Horlock C, Conde B, Villalta O, Porter I (2010) Best-practice IPM strategies for control of major soilborne diseases of vegetable crops. Preliminary final report for VG07125. Department of Primary Industries, Victoria. 227.

Edel V, Steinberg C, Avelang I, Laguerre G, Alabouvette C (1995) Comparison of three molecular methods for the characterisation of *Fusarium oxysporum* strains. *Phytopathology*. **85,** 579-585.

Ehlers JD, Hall AE, Patel PN, Roberts PA, Matthews WC (2000) Registration of 'California Blackeye 27' cowpea. *Crop Science* **40**, 854–855.

Ehlers JD, Sanden BL, Frate CA, Hall AE, Roberts PA (2009) Registration of 'California Blackeye 50' Cowpea. *Journal of Plant Registrations* **3**, 236-240.

Fang MR, Hwang JK (1987) Inheritance of resistance to Fusarium wilt in yardlong bean (*Vigna sesquipedalis* (L.) Fruhw.). *Journal of South China Agricultural University*. **8(1),** 10-13.

Fernanadez D, Assigbetse K, Dubois MP, Geiger JP (1994) Molecular characterisation of races and vegetative compatibility groups in *Fusarium oxysporum* f. sp. *vasinfectum*. *Applied and Environmental Microbiology*. **60,** 4039-4046.

Fernanadez D, Tantaoui A (1994) Random amplified polymorphic DNA (RAPD) analysis: a tool for rapid characterization of *Fusarium oxysporum* f. sp. *albedinis* isolates? *Phytopathology Mediterranean*. **333,** 223-229.

Gal-On A, Wolf D, Antignus Y, Patlis L, Ryu KH, Min BE, Pearlsman M, Lachman O, Gaba V, Wang YZ, Shiboleth YM, Yang J, Zelcer A (2005) Transgenic cucumbers harboring the 54-kDa putative gene of Cucumber fruit mottle mosaic tobamovirus are highly resistant to viral infection and protect non-transgenic scions from soil infection. *Transgenic Research* **14**, 81-93.

Garibaldi A, Baudino M, Minuto A, Gullino ML (2008) Effectiveness of Fumigants and Grafting against Tomato Brown Root Rot Caused by Colletotrichum coccodes. *Phytoparasitica* **36**, 483-488.

Giannakou IO, Karpouzas DG (2003) Evaluation of chemical and integrated strategies as alternatives to methyl bromide for the control of root-knot nematodes in Greece. *Pest Management Science* **59**, 883-892.

Glass NL, Donaldson GC (1995) Development of primer sets for use in PCR to amplify conserved genes from filamentous ascomycetes. *Applied Environmental Microbiology*. **61,** 1323-1330.

Gordon D (1994) Grafting option to overcome bacterial wilt in eggfruit (Queensland Department of Primary Industries, Caboolture) *Queensland Fruit and Vegetable News* **65,** 22-23.

Gordon WL (1965) Pathogenic strains of *Fusarium oxysporum. Canadian Journal of Botany* **43,** 1309-1318.

Gosbee M, Bui K (2001) Snake Bean Fusarium Survey. Technical Annual Report 2000- 2001, Primary Industries, Northern Territory Government. 104-105.

Grattidge R, Obrien RG (1982) Occurence of a 3rd race of Fusarium wilt of tomatoes in Queensland. *Plant Disease* **66**, 165-166.

Gurusamy V, Bett KE, Vandenberg A (2010) Grafting as a tool in common bean breeding. *Canadian Journal of Plant Science* **90**, 299-304.

Ha MT, Huang JW (2007) Control of *Fusarium* wilt of asparagus bean by organic soil amendment and microorganisms. *Plant Pathology Bulletin* **16**, 169-180.

Haglund WA, Kraft JM (1979) Fusarium oxysporum f. sp. pisi, race 6: Occurrence and distribution. *Phytopathology* **69**, 818-820.

Hare WW (1953) A new race of Fusarium causing wilt of cowpea. *Phytopathology,* **43**, 291.

Hare WW (1967) A combination of disease resistant in a new cowpea, Mississippi Silver. *Phytopathology* **57**, 460.

Harris AR (1989) Interactions between Fusarium oxysporum f. sp. tracheiphilum and Meloidogyne spp in Vigna unguiculata. PhD. thesis, University of California, Davis.

Harris AR, Ferris H (1991) Interactions between Fusarium oxysporum f. sp. racheiphilum and Meloidogyne spp. in Vigna unguculata. 2. Specificity of different taxa. *Plant Pathology* **40**(3), 457–464.

Heaton JB, Benson CW (1968) Bacterial wilt, *Pseudomonas solanacearum*, (Erw. Smith 1896) Erw. Smith 1914 of Tomato in the Northern Territory. *The Journal of the Australian Institute of Agriculutural Science* **34**, 37-38.

Helms D, Panella L, Buddenhagen IW, Tucker CL, Gepts PL (1991a) Registration of 'California Blackeye 46' cowpea. *Crop Science* **31**, 1703

Helms D, Panella L, Buddenhagen IW, Tucker CL, Foster KW, Gepts PL (1991b) Registration of 'California Blackeye 48' cowpea. *Crop Science* **31**, 1703-4

Huh Y-C, Woo Y-H, Lee J-M, Om Y-H (2003) Growth and fruit characteristics of watermelon grafted onto Citrullus rootstocks selected for disease resistance. *Journal of the Korean Society for Horticultural Science* **44**, 649-654.

Huitron MV, Diaz M, Dianez F, Camacho F (2007) The effect of various rootstocks on triploid watermelon yield and quality. *Journal of Food Agriculture & Environment* **5**, 344-348.

Hyun JW, Clark CA (1998) Analysis of *Fusarium lateritium* using RAPD and rDNA RFLP techniques. *Mycological Research* **102,** 1259-1264.

Ioannou N (2001) Integrating soil solarization with grafting on resistant rootstocks for management of soil-borne pathogens of eggplant. *Journal of Horticultural Science & Biotechnology* **76**, 396-401.

Jifon JL, Crosby KM, Leskovar DI (2007) Improving the potential for drought and soilborne disease tolerance in muskmelon by grafting. *Hortscience* **42**, 459.

Katan T (1999) Current status of vegetative compatibility groups in *Fusarium oxysporum*. *Phytoparasitica* **27**(1), 51-64.

Katan T, Di Primo E (1999) Current Status of Vegetative Compatibility Groups in *Fusarium oxysporum*: Supplement (1999) *Phytoparasitica* **27(4),** 273-277.

Katan T, Katan J, Gordon TR, Pozniak D (1994) Physiologic races and vegetative compatibility groups of *Fusarium oxysporum* f.sp. *melonis* in Israel. *Phytopathology* **84**,153-157.

Keane PJ, Brown JF (1997) "Disease management: Resistant cultivars", chapter 26 in "Plant Pathogens and Plant Diseases", eds. Brown, J.F. and Ogle, H.J. Rockvale Publications, Armidale, NSW.

Keinath P, Dubose VB, Hassell RL (2010) Lagenaria and Cucurbita Rootstocks Prevent Infection of Watermelon Scions by *Fusarium oxysporum* f. sp *Niveum*. *Hortscience* **45**, 510-511.

King SR, Davis AR (2006) A comparison of novel grafting methods for watermelon in high-wind areas. In 'Proceedings of Cucurbitacea 2006'. (Ed. GJ Holmes) pp. 258-264. (Universal Press: Raleigh, North Carolina).

Kokalis-Burelle N, Bausher MG, Rosskopf EN (2009) Greenhouse evaluation of capsicum rootstocks for mangement of *Meloidogyne incognitia* on grafted bell pepper. *Nematropica* **39**, 121-132.

Koutsika-Sotiriou M, Traka-Mavrona E (2002) The cultivation of grafted melons in Greece, current status and prospects. *Proceedings of the Second Balkan Symposium on Vegetables and Potatoes*, 325-330.

Kubota C, McClure MA, Kokalis-Burelle N, Bausher MG, Rosskopf EN (2008) Vegetable grafting: History, use, and current technology status in North America. *Hortscience* **43**, 1664-1669.

Kurata K (1994) Cultivation of grafted vegetables 2. Development of grafting robots in Japan. *Hortscience* **29**, 240-244.

Lee JM (1994) Cultivation of grafted vegetables 1. Current status, grafting methods, and benefits. *Hortscience* **29**, 235-239.

Lee JM, Bang HJ, Ham HS (1998) Grafting of vegetables. *Journal of Japanese Horticultural Society* **67**, 1098-1104.

Lee JM, Oda M (2003) Grafting of herbaceous vegetable and ornamental crops *Horticultural Reviews* **28**, 61-124.

Levi A, Thies J, Ling K-s, Simmons AM, Kousik C, Hassell R (2009) Genetic diversity among Lagenaria siceraria accessions containing resistance to root-knot nematodes, whiteflies, ZYMV or powdery mildew. *Plant Genetic Resources Characterization and Utilization* **7**.

Liu N, Zhou BL, Zhao X, Lu B, Li YX, Hao J (2009) Grafting Eggplant onto Tomato Rootstock to Suppress Verticillium dahliae Infection: The Effect of Root Exudates. *Hortscience* **44**, 2058-2062.

Lopez-Perez JA, Le Strange M, Kaloshian I, Ploeg AT (2006) Differential response of Mi gene-resistant tomato rootstocks to root-knot nematodes (*Meloidogyne incognita*). *Crop Protection* **25**, 382-388.

M'Hamdi M, Boughalleb N, Ouhaibi N, Tarchoun N, Souli M, Belbahri L (2010) Evaluation of grafting techniques and a new rootstock for resistance of pepper (*Capsicum annuum* L.) towards Phytophthora nicotianae. *Journal of Food Agriculture & Environment* **8**, 135-139.

Manicom BQ, Bar-Joseph M, Kotze JM, Becker MM (1990) A restriction fragment length polymorphism probe relating vegetative compatibility groups and pathogenicity in *Fusarium oxysporum* f. sp. *dianthi. Phytopathology* **80,** 336- 339.

Manulis S, Kogan N, Reuven M, Benyephet Y (1994) Use of RAPD technique for identification of *Fusarium oxysporum* f. sp. *dianthi* from carnation. *Phytopathology* **84,** 98-101.

McDonald BA (1997) The population genetics of fungi: tools and techniques. *Phytopathology* **87,** 448-453.

Mes JJ, van Doom J, Roebroeck EJA, van Egmond E, van Aartrijk J, Boonekamp PM (1994) Restriction fragment length polymorphisms, races and vegetative compatibility groups within a worldwide collection of *Fusarium oxysporum* f. sp. *gladioli*. *Plant Pathology* **43,** 362-370.

Mes JJ, Weststeijn EA, Herlaar F, Lambalk JJM, Wijbrandi J, Haring MA, Cornelissen BJC (1999) Biological and molecular characterisation of *Fusarium oxysporum* f. sp. *lycopersici* divides race 1 isolates into separate virulence groups. *Phytopathology* **89,** 156-160.

Merriman PR, Pywell M, Harrison G, Nancarrow J (1979) Survival of *Sclerotia sclerotiorum* and effects of cultivation practices on disease. *Soil Biology & Biochemistry* **11**, 567-570.

Miguel A (2005a) Use of grafted cucurbits in the Mediterranean region as an alternative to methyl bromide. In 'Proceedings of the VIth International Symposium on Chemical and Non-Chemical Soil and Substrate Disinfestation'. (Ed. A Vanachter) pp. 75-80. (International Society Horticultural Science: Leuven 1).

Miguel A (2005b) Use of grafted plants and IPM methods for the production of tomatoes in the Mediterranean region. In 'Proceedings of the VIth International Symposium on Chemical and Non-Chemical Soil and Substrate Disinfestation'. (Ed. A Vanachter) pp. 141-145. (International Society Horticultural Science: Leuven 1).

Miguel A, Maroto JV, Bautista AS, Baixauli C, Cebolla V, Pascual B, Lopez S, Guardiola JL (2004) The grafting of triploid watermelon is an advantageous alternative to soil fumigation by methyl bromide for control of Fusarium wilt. *Scientia Horticulturae* **103**, 9-17.

Mishra PK, Fox RTV, Culham A (2002) Restriction analysis of PCR amplified nrDNA regions revealed intraspecific variation within populations of *Fusarium culmorum*. *FEMS Microbiology Letters* **215,** 291-296.

MAFES Mississippi Agricultural & Forestry Experiment Station (1980). Mississippi Cream. A new disease resistant Protepea. *MAFES, MSU Research Highlights* **43** (2), 1-3.

Morschel JR (1961) Crop diseases: high rainfall zone - rainfed arable crops. In 'Northern Territory Scientific Liasion Conference, 2-8 February, 1961'. Darwin.

Nagarajan G, Nam MH, Song JY, Yoo SJM, Kim HG (2004) Genetic variation in *Fusarium oxysporum* f.sp. *fragariae* populations based on RAPD and rDNA RFLP analyses. *Plant Pathology Journal* **20(4),** 264-270.

Nelson AJ, Elias KS, Arévalo E, Darlington LC Bailey BA (1997) Genetic characterization of RAPD analysis of isolates of *Fusarium oxysporum* f. sp. *erythroxyli* associated with an emerging epidemic in Peru. *Phytopathology* **87,** 1220-1225.

Nisini PT, Colla G, Granati E, Temperini E, Crino P, Saccardo F (2002) Rootstock resistance to fusarium wilt and effect on fruit yield and quality of two muskmelon cultivars. *Scientia Horticulturae* **93**, 281-288.

Nyvall RF (1999). Field Crop Diseases (3rd edn.). Iowa State University Press. Ames, Iowa. p641.

O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus, *Fusarium* are nonorthologous. *Molecular Phylogenetic Evolution* **7,** 103-106.

O'Donnell K, Guiden C, Sink S, Johnston PR, Crous PW, Glenn A, Riley R, Zitomer NC, Colyer P, Waawijk C, vn der Lee T, Morretti A, Kang S, Kim. H, Geiser DM, Juba JH, Baayen RP, Cromey MG, Bithell S, Sutton DA, Skovgaard K, Plotetz R, Kistler HC, Elliot M, Davis M, Sarver BAJ (2009) A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. *Fungal Genetics and Biology.*, 46.

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences of the United States of America* **95,** 2044-2049.

Oda M (1995) New grafting methods for fruit-bearing vegetables in Japan. *Jarq-Japan Agricultural Research Quarterly* **29**, 187-194.

Oda M (2007) Vegetable seedling grafting in Japan. *IHC 2006: Proceedings of the Xxvii International Horticultural Congress on Global Horticulture: Diversity and Harmony, Introduction*, 175-180.

Ophel-Keller K, McKay A, Hartley D, Herdina, Curran J (2008) Development of a routine DNA-based testing service for soilborne diseases in Australia. *Australasian Plant Pathology* **37**, 243-253.

Orton WA (1902) Some diseases of the cowpea I. The wilt disease of the cowpea and its control. US Department of Agriculture Bureau of Plant Industry Bulletin **17,** 9-20.

Palada M, Wu DL (2005) Tomato production in the hot-wet season using grafting and rainshelter technology. *Hortscience* **40**, 1142.

Palada MC, Wu DL (2007) Increasing off-season tomato production using grafting technology for peri-urban agriculture in Southeast Asia. *Proceedings of the International Conference & Exhibition on Soilless Culture*, 125-131.

Paplomatas EJ, Elena K, Tsagkarakou A, Perdikaris A (2002) Control of verticillium wilt of tomato and cucurbits through grafting of commercial varieties on resistant rootstocks. *Proceedings of the Second Balkan Symposium on Vegetables and Potatoes*, 445-449.

Patiño B, Mirete S, Vázquez C, Jiménez M, Rodríguez MT, González-Jaén MT(2006) Characterization of *Fusarium verticillioides* strains by PCR-RFLP analysis of the intergenic spacer region of the rDNA. *Journal of the Science of Food and Agriculture* **86,** 429-435.

Pavlou GC, Vakalounakis DJ, Ligoxigakis EK (2002) Control of root and stem rot of cucumber, caused by *Fusarium oxysporum* f. sp *radicis-cucumerinum*, by grafting onto resistant rootstocks. *Plant Disease* **86**, 379-382.

Peregrine WTH, Ahmad KB (1982) Brunei: a First Annotated. List of Plant Diseases and Associated Microorganisms. Phytopathological Paper no. 27, Kew, Surrey, England.

Persley DM, O'Brien R, Syme JR (1989) 'Vegetable crops: a disease management guide.' (Department of Primary Industries, Queensland Government: Brisbane).

Piggot TJ, Blackburn KJ (1983) Summary of the Vegetable Variety Trials, Northern Region 1982. Plant Industry Branch, Department of Primary Production, Northern Territory Government. Darwin.

Pitkethley RN (1981) Host range and biotypes of *Pseudomonas solanacearum* in the Northern Territory. *Australiasian Plant Pathlogy* **3**, 46-47.

Poffley M (2003a) Grafting tomatoes for bacterial wilt control (Ed. NTG Horticulture Division) p. 2Darwin).

Poffley M (2003b) Grafting tomatoes for bacterial wilt control. Horticulture Division, Northern Territory Government. Darwin. Agnote B40, 2 pp.

Puhalla JE (1985) Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Can. J. Bot*. **63**, 179-183.

Ramsey MD, Obrien RG, Pegg KG (1992) *Fusarium oxysporum* associated with wilt and root-rot in Queensland - races and vegetative compatibility groups. *Australian Journal of Experimental Agriculture* **32**, 651-655.

Rigert KS (1985). The inheritance of Fusarium wilt resistance in cowpea (*Vigna unguiculata* (L.) Walp.). Thesis (Ph.D.). University of California, Davis OCLC: 80220991.

Rigert KS, Foster KW. (1987). Inheritance of resistance to two races of Fusarium wilt in three cowpea cultivars. *Crop Science*, **27**(2), 220-224.

Rivard CL, Louws FJ (2008) Grafting to Manage Soilborne Diseases in Heirloom Tomato Production. *Hortscience* **43**, 2104-2111.

Rivard CL, Louws FJ, Peet MM, O'Connell S (2008) High tunnels and grafting for disease management in organic tomato production. *Phytopathology* **98**, S133-S133.

Rivard CL, O'Connell S, Peet MM, Louws FJ (2010) Grafting Tomato with Interspecific Rootstock to Manage Diseases Caused by *Sclerotium rolfsii* and Southern Root-Knot Nematode. *Plant Disease* **94**, 1015-1021.

Rodriguez AAC, Menezes M (2005) Identification and pathogenic characterization of endophytic Fusarium species from cowpea seeds. *Mycopathologia* **159**, 79–85.

Sakata Y, Horie H, Ohara T, Kawasaki Y, Sugiyama M (2008a) Influences of rootstock cultivar and storage on the texture of cucumber fruits. *Journal of the Japanese Society for Horticultural Science* **77**, 47-53.

Sakata Y, Ohara T, Sugiyama M (2008b) The history of melon and cucumber grafting in Japan. In 'Proceedings of the International Symposium on Sustainability through Integrated and Organic Horticulture'. (Eds RK Prange, SD Bishop) pp. 217-228. (International Society Horticultural Science: Leuven 1).

Scholefield PB, Blackburn KJ (1985) Horticultural Crops. In 'Agro-research for the semi-arid tropics: North-West Australia'. (Ed. RC Muchow) pp. 192-208. (University of Queensland Press: St Lucia).

Singh KG (1980) A check list of host and disease in Malaysia. Ministry of Agriculture, Kuala Lumpur, Malaysia..

Smith EF (1899) Wilt disease of cotton, watermelon and cowpea (*Neocosmospora* nov. gen.) *US. Dep. Agric. Div. Veg. Physiol. Pathol. Bull*. **17**, 1-54.

Smith FL (1948) California Blackeye 5. California Agriculture, July 1848, p 14.

Smith SN, Helms DM, Temple SR, Frate C (1999) The distribution of Fusarium Wilt of black eyed cowpeas within California caused by *Fusarium oxysporum* f. sp. *tracheiphilum* Race 4. *Plant Disease* **83(7)**,694. (Disease Notes)

Stirling GR, Ashley MG (2003) Incidence of soilborne diseases in Australia's sub-tropical tomato industry. *Australasian Plant Pathology* **32**, 219-222.

Stirling GR, Eden LM, Ashley MG (2004a) Sudden wilt of capsicum in tropical and subtropical Australia: a severe form of Pythium root rot exacerbated by high soil temperatures. *Australasian Plant Pathology* **33**, 357-366.

Stirling GR, Griffin D, Ophel-Keller K, McKay A, Hartley D, Curran J, Stirling AM, Monsour C, Winch J, Hardie B (2004b) Combining an initial risk assessment process with DNA assays to improve prediction of soilborne diseases caused by root-knot nematode (*Meloidogyne* spp.) and *Fusarium oxysporum* f. sp *lycopersici* in the Queensland tomato industry. *Australasian Plant Pathology* **33**, 285-293.

Swanson TA, Van Gundy SD (1985) Influences of temperature and plant age on differentiation of races of Fusarium oxysporum f.sp. tracheiphilum on cowpea. *Plant Disease* **69**(9),779-781.

Taylor M, Bruton B, Fish W, Roberts W (2006) Cost benefit analyses of using grafted watermelons for disease control and the fresh cut market. In 'Cucurbitaceae 2006'. Asheville, North Carolina pp. 277-285.

Taylor M, Bruton B, Fish W, Roberts W (2008) Cost benefit analyses of using grafted watermelon transplants for fusarium wilt disease control. *Proceedings of the Ivth International Symposium on Seed, Transplant and Stand Establishment of Horticultural Crops*, 343-350.

Tesoriero L (2009) Integrated management of greenhouse vegetable diseases: Development of microbial biocontrols, biorational chemical and cultural strategies. NSW Department of Industry and Investment.

Tesoriero LA, Bertus F (2004) Integrated management of greenhouse cucumber and capsicum diseases. Horticulture Australia Ltd. Final Report Project No. VG00069. 65.

Thies JA, Ariss JJ, Hassell RL, Olson S, Kousik CS, Levi A (2010) Grafting watermelon for managment of southern root-knot nematode, *Meloidogyne incognita*, in watermelon. *Plant Disease* **94**, 1195-1199.

Toler RW, Thompson SS, Barber JM (1963) Cowpea (Southern Pea) diseases in Georgia. *Plant Disease Reporter*, **47**(8),746-747.

Traynor M (2003) Tomato grafting. Information sheet IS28. Department of Regional Development, Primary Industry, Fisheries and Resources, Northern Territory Government. Darwin.

Troisi M, Lodovica Gullino M, Garibaldo A (2010) *Gerbera jamesonii*, a new host of *Fusarium oxysporum* f.sp. *tracheiphilum*. *Journal of Phytopathology* **158,** 8-14.

Vakalounakis DJ, Fragkiadakis GA (1999) Genetic diversity of *Fusarium oxysporum* isolates from cucumber: differentiation by pathogenicity, vegetative compatibility, and RAPD fingerprinting. *Phytopathology* **89,** 161-168.

Vawdrey LL, Stirling GR (1996) The use of tolerance and modification of planting times to reduce damage caused by root-knot nematodes (*Meloidogyne* spp) in vegetable cropping systems at Bundaberg, Queensland. *Australasian Plant Pathology* **25**, 240-246.

White TJ, Bruns T, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal DNA genes for phylogenetics. In: PCR protocols: A guide to methods and applications (Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J. Eds). pp: 315-322. Academic Press, New York.

Wicks TJ, Volle D, Baker BT (1978) The effect of soil fumigation and fowl manure on populations of *Fusarium oxysporum* f.sp. *cucumerinum* in glasshouse soil and on the incidence of cucumber wilt. *Agricultural Record* **5**, 4-8.

Woo SL, Zonia A, Del Sorbo G, Lorito M, Nanni B, Scala F, Noviello C (1996) Characterization of *Fusarium oxysporum* f. sp. *phaseoli* by pathogenic races, VCGs, RFLPs and RAPD. *Phytopathology* **86,** 966-973.

Woudt LP, Neuvel A, Sikkema A, Van Grisven MQJM, de Milliano WAJ, Campbell CL, Leslie JF (1995) Genetic variation in *Fusarium oxysporum* from cyclamen. *Phytopathology* **85,** 1348-1355.

Wu D-l, Lin C-h, Sheu Z-m, Palada A (2008a) Selection of Phytophthora blight and bacterial wilt tolerance in sweet pepper lines as rootstocks. *Journal of the Taiwan Society for Horticultural Science* **54**, 47-58.

Wu DL, Palada M, Wang TC (2008b) Developing an integrated crop management system for managing soil-borne diseases in sweet pepper production. *Hortscience* **43**, 1158-1158.

Yetisir H, Sari N, Yucel S (2003) Rootstock resistance to Fusarium wilt and effect on watermelon fruit yield and quality. *Phytoparasitica* **31**, 163-169.

Yli-Mattila T, Paavanen-Huhtala S, Bulat SA, Alekhina IA, Nirenberg HI (2002) Molecular, morphological and phylogenetic analysis of the *Fusarium avenaceum/F. arthrosporioides/F.tricinctum* species complex-a polyphasic approach. *Mycological Research* **106,** 655-669.

Appendix 4.1 Overseas reports of grafting for soilborne pathogen management in vegetable production

Table 4.18: Records of grafting of resistant stocks for pathogen management for selected vegetable crops. Records were largely selected to provide an update on studies since Lee and Oda's (2003) extensive listing of records for tomato from 1942-1997.

Vegetable crop	Pathogen	Successful rootstock species	Reference
Capsicum annuum pepper	*Phytophthora nicotianae*	New *Capsicum annuum* rootstock 'Brutus'	(M'Hamdi *et al.* 2010)
	Meloidogyne incognita	*Capsicum annuum* stocks 'Charleston Hot', 'Carolina Wonder', 'Charleston Belle', 'Mississippi Nemaheart' and 'Carolina Cayenne' were consistently resistant to galling	(Kokalis-Burelle *et al.* 2009)
	Phytophthora capsici and *Ralstonia solanacearum*	*Capsicum annuum* stocks 19 of 124 accessions exhibited very high resistance/	(Wu *et al.* 2008a)
Citrullus lanatus Watermelon	*Fusarium oxysporum* f. sp n*iveum*	Lagenaria and Cucurbita Rootstocks	(Keinath *et al.* 2010)
	Meloidogyne incognita	Best stock PI-296341 -FR *Citrullus lanatus* var *citroides*	(Huitron *et al.* 2007)
	Meloidogyne incognita	Wild *Citrullus lanatus* var *citroides* stocks and one commercial stock	(Thies *et al.* 2010)
	Phytophthora drechsleri	Lagenaria	(Chung *et al.* 2003)
	Fusarium wilt (details not provided)	The most successful stock was 'Shintoza' rootstock (*C. maxima* x *C. moschata*)	(Miguel *et al.* 2004)
	Fusarium oxysporum f.sp. *niveum*	*Cucurbita* or *Lagenaria*	(Taylor *et al.* 2008)
	Fusarium oxysporum f.sp. *niveum* races (0, 1, and 2) o	Stocks tested*: Cucurbita moschate, C. maxima, Lagenaria siceraria*, *Luffa cylindrica*, *Benincasa hispida*, *Lagenaria* hybrids and *Cucurbita* hybrids. *Cucurbita* stocks provide good levels of disease resistance but gave lower quality fruit than melons grafted on some *Lagenaria, Luffa, Benincasa* and commercial watermelon rootstocks	(Yetisir *et al.* 2003)

Cucumis melo Melon	*Fusarium oxysporum* f. sp. *melonis* 1,2	'RS 841', 'P 360', 'ES 99-13' and 'Elsi' were highly resistant both to the races 1 and 2	(Crino *et al.* 2007)
	Fusarium oxysporum f.sp. *melonis*	Multiple field seasons studies found that Cucurbita stocks provided lower levels of disease control in watermelon than *C. maxia x C. moschata*	(Cohen *et al.* 2002).
	Fusarium oxysporum f. sp. melonis 1,2	P360 and PGM 96-05 *Cucumis melo* rootstocks, as well as *Benincasa hispida. Cucumis metuliferus. Cucumis ficifolius, Cucurbita maxima, Cucurbita moschata*, and *Lagenaria siceraria*	(Nisini *et al.* 2002)
	***Monosporascus cannonballus* and Melon necrotic spot virus (MNSV)**	*Cucumis melo/Cucumis melo* stock scions gave better yields than *Cucumis melo*/Cucurbita. Multiple field seasons studies found that Cucurbita stocks provided lower levels of disease control in watermelon than *C. maxia x C. moschata*	(Cohen *et al.* 2004)
Cucumis sativus Cucumber	*Fusarium oxysporum* f. sp. *radicis-cucumerinum*	Cucumber (A27, Cucurbita Ficifolia, Patron F-1., Peto 42.91 F-1, TS- 1358 F-1, and TZ- 148 F-1) were resistant. Peto 42.91 F-1, TS-1358 F-1, and TZ-148 F-1 gave best horticultural performance	(Pavlou *et al.* 2002)
	Verticillium dahliae	Three stocks (two cucumber and one watermelon) tolerant	(Paplomatas *et al.* 2002)
Solanum lycopersicum Tomato	*Sclerotium rolfsii*	Tomato rootstocks Big Power, Beaufort, and Maxifort	(Rivard *et al.* 2010)
	Meloidogyne spp.	Tomato var.s (Beaufort F1, He Man F1, Maxifort)	(Garibaldi *et al.* 2008)
	Colletotrichum coccodes	*Curcibita ficifolia* and *C. maxima x C. moschuta*	(Miguel 2005a)
	Phompsis sclerotiodes	*Solanum lycopersicum* resistant genotypes CRA 66 or Hawaii 7996	(Rivard and Louws 2008)
	Ralstonia solanacearum	*S. lycopersicum x L. hirsutum*	(Miguel 2005b)
	Fusarium oxysporum f.sp. *lycopersici*	3 stocks (unnamed) resistant to race 1 all were susceptible to race 2	(Paplomatas *et al.* 2002)
	Fusarium oxysporum f.sp. *lycopersici* *Verticillium dahliae* race 1 and 2	Eggplant rootstocks increased tomato tolerance to flooding and bacterial wilt disease	(Palada and Wu 2007)
	Ralstonia solanacearum and nematodes (species not listed)	Hawaii 7996 tomato hybrid as rootstocks	(Cardoso *et al.* 2006)
	Ralstonia solanacearum	Tomato BF, LS 89, PFN, PFNT	(Lee 1994a)

Solanum melongena Eggplant	*Verticillium dahliae*	Tomato Rootstock	(Liu *et al.* 2009)
	Verticillium dahliae	*Solanum torvum*.	(Bletsos *et al.* 2002; Bletsos 2006)
	Verticillium dahliae	*Solanum torvum* and *S. sisymbriifolium*	(Arvanitoyannis *et al.* 2005)
	Verticillium dahliae *Meloidogyne incognita*	*Solanum torvum*	(Curuk *et al.* 2009)
	Pyrenochaeta lycopersici *Meloidogyne* spp	Grafting of resistant tomato rootstock 'Brigeor F1' gave protection from *Pyrenochaeta lycopersici* and *Meloidogyne* spp., but partial protection from *Verticillium dahliae*.	(Ioannou 2001)
	Verticillium dahliae		

Appendix 4.2 Estimated costs of snakebean production, yield losses to snakebean wilt, grafting costs and net returns from grafted plants

Table 4.19. Estimated returns ($) for standard yield and a high yielding crop and dollar lost due to reduced production due to Fusarium wilt for planting of a 1000 plants.

	Yield scenario	
Costs[a] and losses	Std. yield	High yield
Planting size	1000	1000
Yield kg/plant	4.5	9.5
Average monthly market price for May – Sept, $ kg	5.25	5.25
Estimated gross return for 1000 plants in absence of Fusarium wilt, $	23625.0	49875.0
Costs for 1000 plants, $		
Transport and box (average $1 per kilogram, actual $9.50 for 10 kg box) $	4500.0	9500.0
Growing costs at $2.50 per plant, $	2500.0	2500.0
Estimated net return for 1000 plants, plants in absence of Fusarium wilt, $	16625.0	37875.0
Estimated net return on three levels of Fusarium infection, $		
Slight disease – 10% production losses	14712.5	33837.5
Moderate disease – 50% production losses	7062.5	17687.5
Severe disease – 100% production losses	-2500.0	-2500.0

[a] Two unknown additional costs were not included: the percentage of the market price commission charged by consolidators and agents; labour for picking and packaging.

Table 4.20. Estimated costs of producing grafted seedlings additional to the costs of producing non-grafted seedlings (alculated for 1000 scion and 1000 rootstock seedlings). Assumes both scion and rootstock seed are self grown by growers.

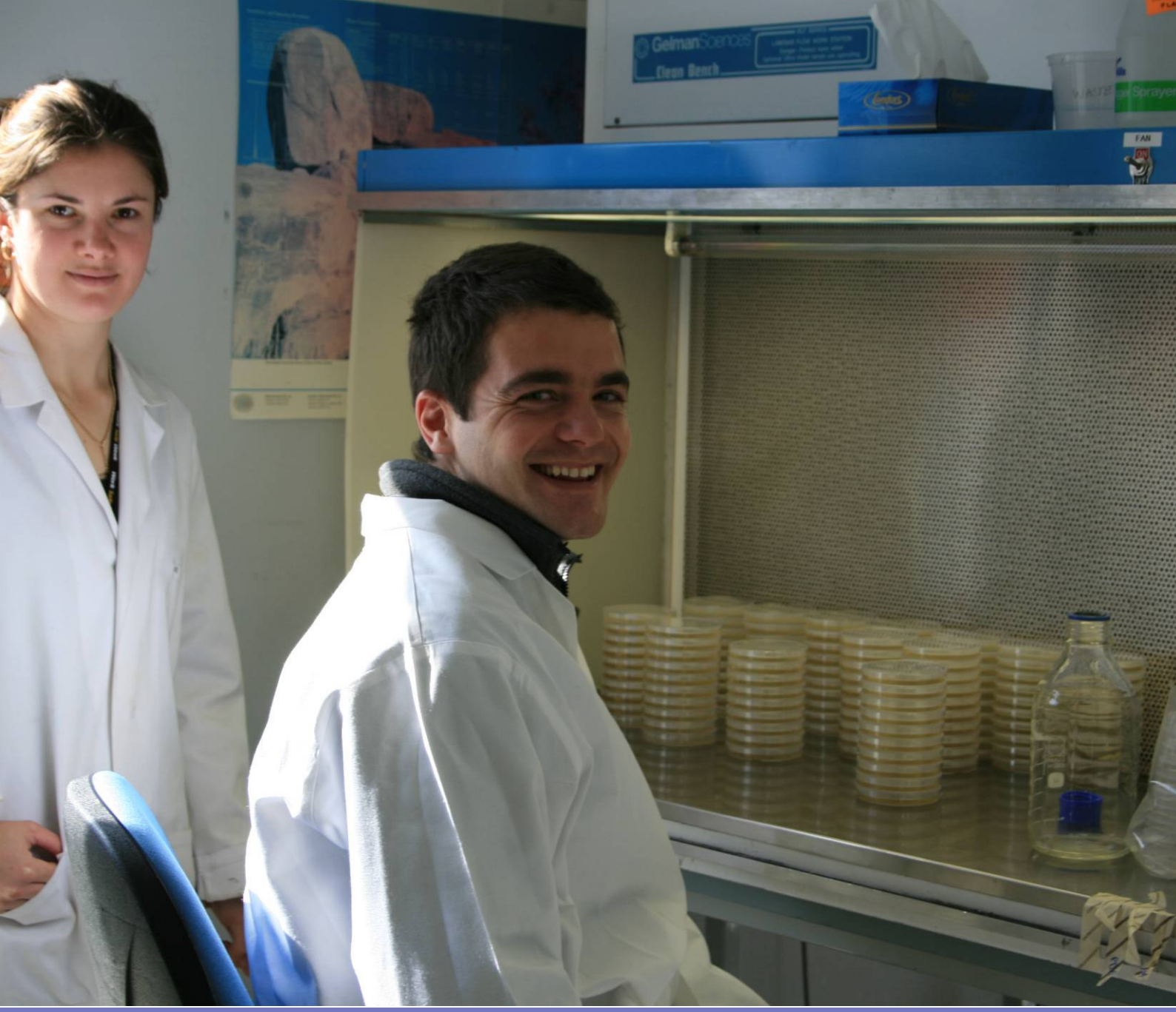
Materials	$
50 x 40 cell trays @ $3.70 ea	185
200 L potting mix @ $25 100 L	50
1000 grafting clips @ $20 200	100
Sub total material costs	$ 335

Labour	hours
Seed collection approx. (hrs)	2
Seed sowing in trays approx.	4
Nursery care approx. 0.5 hrs x 30 days	15
Grafting approx. 1 graft per minute	17
Total hours	38
Cost @ $18.15 (minimum casual rate)	$ 690

Total additional costs of grafting per 1000 plants	$ 1025

Table 4.21. Estimated net returns for average and high yielding snakebean crops after 10, 50 or 100% yield losses (from Table 1) in comparison to net returns from the use of grafted seedlings including grafting costs (from Table 2). Estimates assume no production losses due to Fusarium wilt in grafted plants.

	Yield scenario ($)	
	Std. yield	High yield
Low inoculum and disease, 10% losses	14712.5	33837.5
Low inoculum minus grafting costs	15600.0	36850.0
Additional dollars from grafting	887.5	3012.5
Moderate inoculum and disease, 50% losses	7062.5	17687.5
Moderate inoculum minus grafting costs	15600.0	36850.0
Additional dollars from grafting	8537.5	19162.5
Severe inoculum and disease, 100% losses	-2500.0	-2500.0
Moderate inoculum minus grafting costs	15600.0	36850.0
Additional dollars from grafting	18100.0	39350.0



Plant derived compounds

5. Plant derived compounds

Researchers: Cassie Scoble (Honours candidate),Caroline Donald (supervisor), Ian Porter (supervisor), Oscar Villalta, Denise Wite, David Riches, Department of Primary Industries Victoria,
Kim Plummer (supervisor), La Trobe University.

The potential of plant derived volatile compounds (including essential oils) to control soilborne pathogens was investigated in a series of *in-vitro* (laboratory), pot and field studies. This work was undertaken primarily by Cassie Scoble as her Honours research project. Cassie was assisted in the field component of the work by a number of DPI VIC staff listed above. This work demonstrates:

- inhibitory and biocidal activity of thyme, clove bud and origanum against soilborne pathogens of vegetables *in vitro*.
- disease control potential of thyme, clove bud and origanum in pot bioassay studies at rates of 5 % and 10 % aqueous emulsions.
- inadequate control of soilborne diseases by many of the treatments in the field compared to the soil fumigant Basamid® under moderate to high disease pressure. This is most likely due to the comparatively low concentration of the individual essential oils in commercial products and/or diffusion of the volatile compounds from the soil surface.
- reduced root rot severity in green beans following soil treatment with commercial products Vigor® and ECO-V® at one field site. However, there was no significant effect of these treatments on root rot incidence or on the incidence of lettuce drop (*S. minor*) and clubroot severity (*P. brassicae*) at a second field site.

Further work should be directed towards optimising application rates and exposure times, in order to provide adequate levels of in-field control without causing phytotoxicity. Methods of delivery should also be addressed to retain the volatile components of the products used within the soil for as long as possible to improve efficacy but also to minimise human exposure to these odorous compounds.

5.1 Introduction

Plant-derived compounds are produced by plants as secondary metabolites, some of which have been identified as having strong antibacterial and antifungal activity (Kalemba and Kunicka, 2003). Secondary metabolites with antimicrobial activity include alkaloids, phenolics and polyphenolics, lectins, polypeptides and terpenes (Cowan, 1999). Essential oils are a major group of plant- derived products. The antimicrobial activity of some essential oils has been clearly demonstrated *in vitro* against a range of pathogens including *Rhizoctonia solani, Pythium ultimum, Fusarium solani, Colletotrichum lindemuthianum* (Zambonelli *et al*. 1996), *Sclerotium cepivorum* (Montes-Belmont and Prados-Ligero 2006), *Sclerotinia sclerotiorum, Rhizopus stolonifer* and *Mucor* sp. (Edris and Farrag 2003), *Botrytis cinerea* (Wilson *et al*. 1997) and *Phytopthora infestans* (Soylu *et al.* 2006). This activity has been linked to phenolic and terpenoid compounds contained in the essential oils such as carvacrol, eugenol, geraniol and thymol, which also show high antimicrobial activity in pure form. Zambonelli *et al.* (1996) attributed the strong fungicidal activity of thyme oil (*Thymus vulgaris*) against

← Image previous page, chapter 5 coversheet – researchers Cassie Scoble and Jorge Ghiano conducting laboratory based volatile bioassays.

Rhizoctonia solani, Fusarium solani, and other pathogenic fungi to the active compound thymol. Similarly Tullio *et al*. (2007) concluded that the bioactivity of thyme and clove oil against filamentous fungi was due to phenolic compounds thymol, carvacrol and eugenol. This chapter reports the antimicrobial activity of essential oils and other plant-derived products against important soilborne pathogens (*Pythium, Fusarium, Rhizoctonia* and *Sclerotinia* spp.) which affect vegetable crops, and evaluates their efficacy and potential for disease control in soil.

This work was conducted in three stages:

(1) Dose-dependent *in-vitro* experiments were performed to determine the antimicrobial activity of all treatments against 4 pathogens. Since previous studies have shown that plant volatiles have different efficacies when tested in contact and vapour phases (Tullio *et al.* 2007; Soylu *et al,* 2007), methods were used to screen both phases of all of the plant derived compounds and actives used.

(2) Promising treatments from *in-vitro* studies were selected and their efficacy optimised to identify lowest concentrations that inhibit growth (protectant potential) or kill inoculum (biofumigant potential).

(3) The potential for disease control was determined in pot bioassays and field experiments.

5.2 *In-vitro* (laboratory) screening to evaluate antimicrobial activity

5.2.1 Materials and methods

Treatments

Initial screening was carried out to identify plant-derived treatments with antimicrobial activity against 4 important pathogens of vegetable crops (*Pythium, Fusarium, Rhizoctonia* and *Sclerotinia* spp). Screening was done using *in vitro* bioassays to test the antimicrobial activity of 21 plant-derived volatiles (comprising of essential oils, chemical compounds of essential oils, and products of plant-volatile blends) against pathogen mycelium. Treatments tested were:

- 15 essential oils (Table 5.1) (purchased from Sydney Essential Oils Company)
- 4 purified compounds (Sigma®) which are major constituents of some essential oils (terpenoid compounds thymol, carvacrol and eugenol, and a phenolic compound geraniol)
- 2 commercial products of plant volatile blends; ECO-V® (mustard oil and allyl isothiocynates) and Vigor® (mustard and chilli pepper extracts)

Table 5.1. Plant essential oils tested for antimicrobial activity against 4 pathogens and 1 biological control agent using vapour and contact phase methods

FAMILY	SCIENTIFIC NAME	COMMON NAME	COUNTRY OF ORIGIN	PLANT PART	METHOD OF EXTRACTION
APIACEAE	*Foeniculum vulgare dulce*	Fennel Sweet	Australia	Seed	Steam distilled
GERANIACEAE	*Pelagonium graveolens*	Geranium	Egypt	Flowering plant	Steam distilled
LABIATEAE	*Ocimum basilicum*	Basil CT linalool	India	Flowering heads	Steam distilled
	Mentha piperita	Peppermint	Vietnam	Flowering herb	Steam distilled
	Rosmarinus officinalis	Rosemary	Tunisia	Flowering tops	Steam distilled
	Thymus vulgaris	Thyme	France	Flowering tops/ leaves	Steam distilled
	Origanum vulgare	Origanum	Spain	Dried herb	Steam distilled
MYRTACEAE	*Backhousia citriodora*	Lemon myrtle	Australia	Whole plant	Steam distilled
	Eugenia caryophyllata	Clove bud	Sri Lanka	Buds	Steam distilled
	Eucalyptus radiata	Eucalyptus Australiana	Australia	Leaves and branches	Steam distilled
	Melaleuca alternifolia	Tea tree	Australia	Whole plant	Steam distilled
PINACEAE	*Pinus massoniana*	Pine	China	Needles/ branches	Steam distilled
PIPERACEAE	*Piper nigrum*	Black pepper	India	Dried fruit	Steam distilled
RUTACEAE	*Citrus sinensis*	Orange Sweet	Australia	Peel	Cold pressed
ZINGIBERACEAE	*Elettaria cardamomum*	Cardamom	Sri Lanka	Pod	Steam distilled

Micro-organisms and media

The treatments were tested against *Pythium irregulare, P. aphanidermatum, P.sulcatum, Rhizoctonia solani* and *Fusarium oxysporum* in contact bioassays and against *P. irregulare, R. solani, F. oxysporum, Sclerotinia minor* and *Trichoderma hamatum* (biological control agent) in vapour phase bioassays. The optimising bioassays tested treatments against *Pythium dissotocum* (a member of this species complex isolated from green beans), *F. oxysporum, R. solani, S. minor* and *T. hamatum* (Table 5.2). Potato dextrose agar (PDA) (Oxoid®) (39 grams per 1 L dionised water) was used for maintaining and growing *Rhizoctonia, Fusarium and Sclerotinia* isolates. A selective V8 media (48 mL V8 juice in 1 L deionised water adjusted to pH 6.5) was used for maintaining and growing *Pythium* isolates. All *in-vitro* bioassays were conducted on PDA media. A commercial biological product (1 gram Lettuce mate® in 9 mL sterile distilled water, 0.5 mL spread on PDA) was used to grow colonies of *T. hamatum* which were then sub-cultured onto PDA before use in the bioassays.

Table 5.2. Isolates of micro-organisms of which plant volatiles were tested against for antimicrobial activity using vapour and contact phase methods

Test organism	Species	Host	Location/source
Pythium (pathogen)	*P. irregulare*	Parsley	Stanthorpe, Qld (ex. Des Auer, DPI VIC)
	P. aphanidermatum	Spinach	Unknown, WA (ex. Herbarium VPRI 32634 a)
	P. sulcatum	Parsley	Lang Lang, Vic (ex. Des Auer, DPI VIC)
	P. dissotocum	Beans	Lindenow, Vic
Fusarium (pathogen)	*F. oxysporum*	Capsicum	Werribee South Vic (ex Herbarium VPRI 13039)
Sclerotinia (pathogen)	*S. minor*	Lettuce	Boneo, Victoria
Rhizoctonia (pathogen)	*R. solani*	Broccoli	Boneo, Victoria
Trichoderma (biological control)	*T. hamatum*	Product (Lettuce Mate®)	

Contact bioassays

In the contact phase, treatments were added to PDA to determine their effect on pathogen growth. Treatments were added to the media immediately before being poured (40-45ºC) into petri dishes at concentrations of 500, 1000 and 2500 ppm (final concentrations equivalent to 25, 50 and 125µL per plate). After the media had set, the centre of the plates were inoculated with a plug of agar with mycelium (5mm diameter) taken from the growing margin of cultures (7 days growth) of isolates of *P. sulcatum, P. irregulare, P. aphanidermatum, F. oxysporum* and *R. solani*. After inoculation plates were incubated at room temperature (range 17-20ºC). To determine the efficacy of treatments for the inhibition of pathogen growth, sequential inspections (from 1-10 days depending on the growth rate of the specific test pathogen) measuring colony diameter (mm) of mycelium were taken until the growth of control plates had reached the plates edge (85mm). For analysis, colony measurements excluding the agar plug (5mm) were used. Treatments were tested on four replicate plates.

Biocidal activity (whether mycelium had been killed by the treatment) was tested by replating plugs with suppressed growth or with no growth onto unamended PDA. If growth was not resumed within 10 days, the treatment was considered biocidal to the isolate.

Vapour phase bioassays

In the vapour phase, treatments were tested using the technique by Lee *et al.* (2009). PDA plates were prepared using plastic petri dishes. Mycelial agar plugs of *P. irregulare, F. oxysorum, R. solani, S. minor* and soil beneficial *T. hamatum* were placed in the centre of the agar. The lid of each plate was inverted and a sterile filter paper disc (Whattman no. 1) was attached to the (inside) centre of the lid. An aliquot of treatment, 1, 5, or 10 µL per plate (equivalent to 22.7, 113.5 & 227 µL 1^{-1} in air) was added to the paper discs. The lids were replaced on the agar plates and sealed, then wrapped in foil and placed in sealable plastic tubs to prevent leaking and mixing effects of vapours. Plates were stored at

room temperature (17-20ºC). Diameter of mycelial colonies was measured to determine treatment effect on growth as described earlier in the contact method. Pathogens were incubated on PDA with treatment- free paper discs as controls. All treatments were replicated four times.

Optimisation to identify lowest concentrations which inhibit and kill mycelium

The most effective treatments identified in the screening bioassays were tested further at reduced rates using contact bioassays. Treatments which completely inhibited growth and were biocidal at the lowest rate tested in the screening bioassays were tested at 1, 10, 100 and 500 ppm per plate (equivalent to 0.05, 0.5, 5 & 25µL per plate). Four essential oils (clove bud, thyme, origanum and lemon myrtle) and four compounds (thymol, geraniol, carvacrol and geraniol) were compared to plant volatile products (Vigor® and ECO-V®). Commercial products based on the four most effective essential oils were sought. Promax™ (Bio HumaNetics) containing thyme oil was included in optimisation studies. Treatments such as essential oils do not mix well in water; therefore all treatments were mixed with an emulsifier. Preliminary bioassays confirmed that Tween 80 (Sigma®) at 0.5, 1 and 2 % had no significant effect on mycelium growth. Thus in the optimising bioassays, Tween 80 (1 %) was used to improve oil solubility in the agar. Aliquots of treatments were added to PDA immediately before being poured (40-45ºC) into petri dishes. Once the media had set, plates were inoculated with an agar plug of mycelium (7 days growth) of isolates of key pathogens (*P. dissotocum, F. oxysporum, R. solani* and *S. minor*). The biocontrol agent *T. hamatum*, a common soil fungus, was included to evaluate the effects of plant-derived volatiles on this beneficial soil organism. Colony growth and biocidal activity were recorded as described previously.

Statistical analysis of data from in-vitro studies

Mycelial growth data were analysed using analysis of variance (ANOVA, Genstat 12th Edition). Data were transformed as required before analysis to normalise the variance and LSD tests (5 % level) were used to compare treatment means and treatment effects. Colony inhibition compared to untreated controls was calculated (100- diameter of control - diameter of treated / diameter of control*100) (Pandey *et al*. 1982). Standard error of the means (SEM) was calculated (standard deviation / square root of no. reps).

5.2.2 Results

Antimicrobial activity of plant volatiles in contact phase

The 4 chemical compound treatments eugenol, thymol, carvacrol and geraniol showed broad-spectrum antimicrobial activity against all the test pathogens. All 4 compounds completely inhibited mycelial growth and were biocidal at all rates tested (500, 1000 and 2500 ppm) against *P. irregulare, P. aphanidermatum, P. sulcatum, R. solani* and *F. oxysporum* (data not shown).

Of the 14 essential oils tested, seven inhibited mycelial growth and showed biocidal activity. Three oils showed growth inhibition and biocidal activity against all of the test pathogens. Origanum, clove bud and thyme completely inhibited growth of all pathogens *(R. solani, F. oxysporum, P. irregulare, P. aphanidermatum* and *P. sulcatum*) at 500, 1000 and 2500 ppm (Table 5.3). Origanum was biocidal to *R. solani, P. irregulare, P. sulcatum*, and *P. aphanidermatum* at the lowest rate tested of 500 ppm. Clove bud was biocidal to *R. solani, P. sulcatum and P. aphanidermatum* at 500 ppm and became biocidal to *F. oxysporum* and *P. irregulare* at 1000 ppm. Thyme was biocidal to *R. solani* and *P. sulcatum* at 500 ppm, and became biocidal to *F. oxysporum, P. irregulare* and *P. aphanidermatum* at 1000 ppm (Table 5.3).

Geranium inhibited the growth *of R. solani, P. irregulare, P. sulcatum*, and *P. aphanidermatum* at 500 ppm, and completely inhibited growth of all pathogens at 1000 ppm. Geranium was biocidal to *R. solani* at 1000 ppm. Bioweed™ inhibited growth of all pathogens at 2500 ppm and was biocidal to *R. solani, P. sulcatum*, and *P. aphanidermatum* at this rate. Peppermint completely inhibited growth of all pathogens except *F. oxysporum* at 2500 ppm, and similar to pine was biocidal to *R. solani, P. sulcatum*, *and P. aphanidermatum* at this rate. Basil completely inhibited growth and was biocidal *to R. solani* at 2500 ppm. Tea tree completely inhibited growth of *R. solani* and *F. oxysporum* and was biocidal to *R. solani* at 2500 ppm.

When comparing the most effective treatments with commercial products Vigor® (dazitol) and ECO-V®, the commercial products were not as effective at reducing growth and killing pathogen mycelium. ECO-V® completely inhibited growth of *R. solani* at 1000 and 2500 ppm and was biocidal at 2500 ppm only (Table 5.4). Vigor® completely inhibited growth and was biocidal to *R. solani* at 2500 ppm but at rates below 2500 ppm the colonies grew to the edge. Vigor® significantly (P=0.001) reduced mycelial growth of *F. oxysporum* at 2500 ppm but mycelium grew normally on fresh PDA (Table 5.4). ECO-V® significantly (P=0.001) reduced growth of *F. oxysporum* at 1000 and 2500 ppm but was not biocidal (Table 5.4). There was a significant interaction between the concentration of Vigor® and ECO-V® and mycelial growth of *F. oxysporum*.

Table 5.3. Summary of results from first series of 5 tests showing the antimicrobial activity at high rates of 14 essential oils tested in contact bioassays

		Percent mycelial growth compared to control				
	Dosage (ppm)	*R. solani*	*F. oxysporum*	*P. irregulare*	*P. sulcatum*	*P. aphanidermatum*
Origanum[a]	**500**	0.0*	0.0	0.0*	0.0*	0.0*
	1000	0.0*	0.0*	0.0*	0.0*	0.0*
	2500	0.0*	0.0*	0.0*	0.0*	0.0*
Clove bud[a]	**500**	0.0*	0.0	0.0	0.0*	0.0*
	1000	0.0*	0.0*	0.0*	0.0*	0.0*
	2500	0.0*	0.0*	nt	nt	nt
Thyme[a]	**500**	0.0*	0.0	0.0	0.0*	0.0
	1000	0.0*	0.0*	0.0*	0.0*	0.0*
	2500	0.0*	0.0*	nt	nt	nt
Geranium[a]	**500**	40.0	53.8	31.9	20.3	35.9
	1000	0.0*	0.0	0.0	0.0	0.0
	2500	0.0*	0.0	nt	nt	nt
Pine[a]	**500**	71.6	29.1	100.0	72.5	100.0
	1000	14.9	0.0	11.3	1.3	22.2
	2500	0.0*	0.0	0.0	0.0*	0.0*
Peppermint	**500**	100.0	78.1	100.0	100.0	100.0
	1000	51.9	41.4	47.5	0.0	22.2
	2500	0.0*	29.5	0.0	0.0*	0.0*
Basil	**500**	100.0	79.3	100.0	89.1	100.0
	1000	28.8	59.8	9.7	16.9	13.5
	2500	0.0*	15.1	nt	nt	nt
Tea tree[a]	**500**	86.2	100.0	100.0	50.6	100.0
	1000	56.0	0.0	45.0	26.3	62.8
	2500	0.0*	0.0	nt	nt	nt
Fennel Sweet	**500**	75.0	84.1	100.0	100.0	100.0
	1000	20.6	74.9	100.0	100.0	100.0
	2500	0.0	37.9	100.0	100.0	100.0
Cardamom	**500**	100.0	75.7	100.0	100.0	100.0
	1000	89.4	100.0	100.0	76.6	100.0
	2500	17.8	39.4	nt	nt	nt
Eucalyptus	**500**	100.0	95.6	100.0	89.4	100.0
	1000	100.0	89.6	100.0	100.0	100.0
	2500	100.0	77.7	nt	nt	nt
Rosemary[a]	**500**	98.6	100.0	100.0	100.0	100.0
	1000	93.8	100.0	100.0	100.0	100.0
	2500	86.9	46.6	100.0	100.0	100.0
Black pepper	**500**	100.0	94.8	100.0	100.0	100.0
	1000	100.0	90.8	100.0	100.0	100.0
	2500	100.0	84.1	nt	nt	nt
Orange Sweet	**500**	100.0	100.0	100.0	100.0	100.0
	1000	100.0	97.2	100.0	100.0	100.0
	2500	100.0	88.5	nt	nt	nt

nt= not tested, * = biocidal (no mycelial growth observed after plugs were transferred to fresh PDA media)

[a] = growth at 8 days (other treaments at 9 days)

Table 5.4. Antimicrobial activity of plant volatile products on the growth of 2 pathogens at 10 days incubation in contact bioassays.

Treatment	Rate (ppm)	Mean colony growth *R. solani*[2]	Mean colony growth *F. oxysporum*	% growth[1] *R.solani*[2]	% growth[1] *F.oxysporum*
Vigor®	500	80.0 ± 0.0	80.0 ± 0.0	100.0	100.0
	1000	80.0 ± 0.0	71.3 ± 0.3	100.0	89.1
	2500	0.0 ± 0.0*	50.5 ± 3.2	0.0	63.1
ECO-V®	500	80.0 ± 0.0	70.8 ± 0.4	100.0	88.4
	1000	0.0 ± 0.0	57.8 ± 1.9	0.0	72.2
	2500	0.0 ± 0.0*	14.3 ± 2.8	0.0	17.8
Untreated control	0	80.0 ± 0.0	80.0 ± 0.0		
F- test			0.001		0.001
Lsd			9.4		11.8

* Biocidal activity. +/- SEM

[1] = % growth compared to untreated control

[2] = Treatments were either completely inhibitory or completely ineffective against R. solani. There was no variation in the data so statical analysis is not possible (and not necessary).

Antimicrobial activity of treatments in vapour phase

Of the 19 treatments tested, 5 treatments showed biocidal activity in the vapour phase including 3 essential oils (origanum, thyme and clove bud), chemical compound eugenol and commercial product ECO-V®. Origanum completely inhibited mycelial growth of all test pathogens (*R. solani, F. oxysporum, P. irregulare* and *S. minor*) and the beneficial soil fungi *T. hamatum* at all concentrations tested after 7 days incubation, except for *S.minor* which was not inhibited at the lowest concentration (Table 5.5). Origanum and thyme were biocidal to all pathogens tested as well as *T. hamatum* at the highest concentration of 10µL. Origanum was biocidal to *R. solani, P. irregulare* and *S. minor* and thyme was biocidal to *R. solani* and *F. oxysporum* at 5µL. Origanum was the only treatment which showed biocidal activity at the lowest concentration and was efficacious against resilient pathogens *S. minor* and *R. solani* (Table 5.5).

Several treatments including geranium, pine, sweet fennel, basil, tea tree and peppermint and the compound geraniol showed biostatic activity (Tables 5.5 & 5.6). These treatments either inhibited colony growth or completely suppressed growth of some test organisms, but the colonies resumed growth when plated onto fresh agar. Geranium had no effect on growth of *R. solani* or *S. minor,* but did have a positive dose dependent response against *F. oxysporum, P. irregulare* and *T. hamatum*. Pine had a positive dose dependent response against *R. solani, F. oxysorum* and *P. irregulare* as did sweet fennel and basil against *P. irregulare*. Tea tree had a positive dose dependent response effect against *R. solani* as did peppermint against *F. oxysporum* and *P. irregulare.*

The chemical compound eugenol completely inhibited mycelial growth of *F. oxysporum, P. irregulare* and *T. hamatum* at all concentrations. Eugenol was biocidal to *F. oxysporum* at 5µL and biocidal to *P. irregulare* at 1 and 10 µL but did not kill *T. hamatum.* Eugenol had no effect on mycelial growth of *R. solani* or *S. minor* (Table 5.5). Carvacrol completely inhibited the growth of *R. solani* at all concentrations, yet this activity was biostatic as mycelium grew once replated on fresh media (Table 5.6). Geraniol had no effect on growth of *S. minor* and while it inhibited growth of *R. solani, F. oxysporum, P. irregulare* and *T. hamatum,* the effects were not dose-dependent (Table 5.5).

Of the commercial products tested, ECO-V® was most the effective and Vigor® was ineffective at inhibiting mycelial growth (Tables 5.5 & 5.6). ECO-V® completely inhibited growth of *P. irregulare* at all concentrations and was biocidal at 5 and 10 µL. ECO-V® had a positive dose-dependant effect against *F. oxysporum* and was biocidal at the highest concentration of 10µL. ECO-V® had no effect on *R. solani* but did have a positive dose-dependant response against *F. oxysporum, S. minor* and *T. hamatum* (Table 5.5).

Table 5.5. Summary of results from 2 tests showing the antimicrobial activity of 10 plant volatile treatments tested *in vitro* using vapour bioassays at 7 days incubation.

		% growth compared to control				
Treatment	**Dosage (µL/plate)**	***R. solani***	***F. oxysporum***	***P. irregulare***	***S. minor***	***T. hamatum***
Origanum	**1**	0.0	0.0	0.0*	100.0	0.0
	5	0.0*	0.0	0.0*	0.0*	0.0
	10	0.0*	0.0*	0.0*	0.0*	0.0*
Clove bud	**1**	15.3	0.0	0.0	100.0	33.3
	5	10.9	0.0	0.0*	80.2	11.0
	10	0.0	0.0*	0.0*	72.0	0.0
Thyme	**1**	76.5	0.0	57.3	100.0	5.4
	5	0.0*	0.0*	0.0	11.1	0.0
	10	0.0*	0.0*	0.0*	0.0*	0.0*
Geranium	**1**	100.0	83.3	66.7	100.0	86.5
	5	100.0	25.1	0.0	100.0	31.6
	10	100.0	0.0	0.0	100.0	0.0
Pine	**1**	100.0	104.6	100.0	100.0	100.0
	5	81.4	71.4	82.3	100.0	100.0
	10	33.3	30.8	0.0	100.0	60.7
Rosemary	**1**	100.0	116.8	100.0	100.0	100.0
	5	100.0	138.1	100.0	100.0	100.0
	10	100.0	130.0	100.0	100.0	100.0
Black pepper	**1**	100.0	94.0	100.0	100.0	100.0
	5	100.0	95.8	100.0	100.0	100.0
	10	100.0	94.9	100.0	100.0	100.0
ECO-V	**1**	100.0	117.6	0.0	94.8	100.0
	5	100.0	63.0	0.0*	56.2	0.0
	10	100.0	0.0*	0.0*	27.0	0.0
Geraniol	**1**	46.9	7.8	0.0	100.0	14.0
	5	46.9	13.8	31.3	100.0	13.8
	10	46.9	0.0	15.6	100.0	0.0
Eugenol	**1**	100.0	0.0	0.0*	100.0	0.0
	5	100.0	0.0*	0.0	100.0	0.0
	10	100.0	0.0	0.0*	100.0	0.0

* Biocidal (no mycelial growth observed after plug transferred to fresh PDA media)

Table 5.6. Summary of results from 2 tests showing the antimicrobial activity of 9 plant volatile treatments tested *in vitro* using vapour bioassays at 7 days incubation.

Treatment	Dosage (ppm)	% growth compared to control				
		R. solani	*F. oxysporum*	*P. irregulare*	*S. minor*	*T. hamatum*
Sweet fennel	**1**	100.0	98.7	100.0	100.0	100.0
	5	100.0	83.8	51.2	100.0	100.0
	10	100.0	52.5	0.0	100.0	44.1
Basil	**1**	100.0	90.5	100.0	100.0	93.3
	5	100.0	79.2	51.2	100.0	93.4
	10	22.0	23.4	0.0	37.6	39.2
Tea tree	**1**	100.0	91.5	100.0	100.0	100.0
	5	38.7	92.8	100.0	100.0	100.0
	10	0.0	69.5	0.0	100.0	100.0
Orange sweet	**1**	100.0	107.0	100.0	100.0	100.0
	5	100.0	103.6	100.0	100.0	100.0
	10	100.0	94.2	100.0	100.0	100.0
Peppermint	**1**	100.0	86.2	34.3	100.0	100.0
	5	100.0	71.2	28.3	100.0	96.6
	10	8.8	14.0	5.6	100.0	17.4
Eucalyptus	**1**	100.0	105.2	100.0	100.0	100.0
	5	100.0	103.3	100.0	100.0	100.0
	10	100.0	80.1	100.0	100.0	100.0
Cardamom	**1**	100.0	84.5	100.0	100.0	100.0
	5	100.0	79.5	100.0	100.0	100.0
	10	100.0	46.2	90.5	100.0	94.8
Vigor®	**1**	100.0	103.9	100.0	100.0	100.0
	5	100.0	95.6	100.0	100.0	100.0
	10	100.0	60.0	76.9	100.0	76.9
Carvacrol	**1**	0.0	91.8	100.0	100.0	100.0
	5	0.0	100.0	100.0	36.8	100.0
	10	0.0	87.4	100.0	15.6	100.0

Optimisation of treatments with antimicrobial potential

All treatments showed biocidal activity against the test organisms except for commercial products Vigor®, ECO-V® and Promax™. Of the chemical compounds tested, thymol completely inhibited growth of *F. oxysporum, S. minor* and *P. dissotocum* at 100 ppm and was biocidal to all test organisms at 500 ppm (Table 5.7). Thymol also significantly ($P< 0.001$) reduced mycelial growth of all organisms at 10 ppm compared to the untreated controls. Carvacrol completely inhibited growth of *P. dissotocum* and *R. solani* at 100 ppm and was biocidal against all test organisms except to *T. hamatum* at 500 ppm. Carvacrol reduced ($P< 0.001$) mycelial growth of all organisms except *S. minor* at 10 ppm (Table 5.8). Eugenol completely inhibited growth of *F. oxysporum, R. solani* and *P. dissotocum* at 500 ppm and significantly ($P< 0.001$) reduced growth of *F. oxysporum* and *R. solani* at 1, 10 and 100 ppm (Table 5.8).

Origanum completely inhibited growth of *F. oxysporum, R. solani*, *P. dissotocum, S. minor* and *T. hamatum* at 500 ppm. Origanum, thyme and clove bud essential oils completely inhibited mycelial growth of all 4 pathogens at 500 ppm. Origanum and clove bud were biocidal against *S. minor* and *R. solani*, and thyme was biocial to *P. dissotocum* at 500 ppm (Tables 5.7 & 5.8).

Table 5.7. Summary of results from 6 tests showing the mycelial growth and biocidal activity of selected treatments at reduced rates of 1, 10, 100 and 500 ppm against 4 isolates of pathogen and 1 soil biological. Treatments tested *in vitro* using the contact method with the addition of Tween 80 at 1 %.

	Rate ppm	TWN 1%	*R. solani* (4 days)	*F. oxysporum* (4 days)	*S. minor* (2 days)	*P. dissotocum* (3 days)	*T. hamatum* (2 days)
Essential oil	1	+	72.4 ± 1.1	26.8 ± 1.0	57.7 ± 2.3	32.2 ± 16.2	48.4 ± 2.5
Thyme	10	+	68.3 ± 0.8	26.0 ± 0.5	57.5 ± 1.3	50.2 ± 9.4	44.3 ± 1.6
	100	+	4.5 ± 1.2	5.1 ± 0.2	41.1 ± 2.1	0.0	14.6 ± 1.0
	500	+	0.0	0.0	0.0	0.0*	3.4 ± 0.4
	500	+	0.0	0.0	0.0	0.0*	0.0
Clove bud	1	+	61.5 ± 3.8	21.2 ± 0.5	58.6 ± 1.0	6.5 ± 0.9	53.6 ± 3.3
	10	+	50.0 ± 0.7	21.4 ± 0.8	52.1 ± 1.5	2.8 ± 1.0	57.8 ± 0.4
	100	+	13.0 ± 1.3	7.3 ± 0.3	39.8 ± 3.4	0.0	28.5 ± 4.9
	500	+	0.0	0.0	0.0*	0.0	0.0
	500	-	0.0*	0.0	0.0*	0.0	0.0
Compound	1	+	70.2 ± 0.6	27.5 ± 0.1	61.3 ± 0.5	38.4 ± 15.5	49.0 ± 1.9
Thymol	10	+	67.7 ± 1.6	21.2 ± 1.0	41.9 ± 1.3	1.0 ± 1.0	16.9 ± 0.9
	100	+	8.2 ± 3.9	0.0	0.0	0.0	5.9 ± 0.3
	500	+	0.0*	0.0*	0.0*	0.0*	0.0*
	500	-	0.0*	0.0	0.0*	0.0*	0.0*
Geraniol	1	+	73.8 ± 1.2	25.5 ± 1.2	65.5 ± 0.2	48.3 ± 18.6	46.0 ± 1.8
	10	+	67.5 ± 1.1	27.7 ± 0.1	62.0 ± 0.6	58.2 ± 19.5	42.6 ± 0.7
	100	+	56.9 ± 0.8	18.9 ± 0.4	42.7 ± 2.5	0.0	30.4 ± 0.9
	500	+	7.5 ± 3.6	5.3 ± 1.8	0.0*	0.0*	3.1 ± 0.3
	500	-	15.5 ± 0.5	4.0 ± 1.4	0.0	0.0*	4.4 ± 1.8
Product	100	+	76.5 ± 1.0	28.7 ± 0.3	58.0 ± 1.3	nt	49.9 ± 1.5
Promax®	500	+	76.2 ± 0.9	28.1 ± 0.4	59.7 ± 2.1	nt	50.0 ± 1.2
	500	-	73.3 ± 0.7	27.8 ± 0.5	62.4 ± 1.2	nt	42.8 ± 1.4
ECO-V	1	+	72.1 ± 2.7	25.3 ± 1.4	58.3 ± 1.4	62.8 ± 17.2	58.6 ± 2.6
	10	+	72.9 ± 2.3	27.4 ± 0.7	57.5 ± 2.8	30.9 ± 16.7	53.9 ± 2.0
	100	+	71.0 ± 1.5	29.1 ± 0.1	52.8 ± 1.4	80.0 ± 0.0	54.6 ± 1.6
	500	+	65.1 ± 1.5	27.1 ± 0.1	46.5 ± 1.1	46.8 ± 19.2	49.1 ± 1.3
	500	-	65.0 ± 1.5	28.6 ± 0.2	53.1 ± 1.2	80.0	51.7 ± 0.6
Untreated control	0		**73.3 ± 1.6**	**28.8 ± 0.3**	**59.6 ± 2.5**	**80.0**	**52.6 ± 0.6**

Mycelial colony diameter (mm) +/- SEM. 0= no growth observed, 80= colony grown to edge of plate
* biocidal activity (no mycelial growth observed after plugs were transferred to fresh PDA media)

Table 5.8. Summary of results from 6 tests showing the mycelial growth and biocidal activity of selected treatments at reduced rates of 1, 10, 100 and 500 ppm on 4 isolates of pathogen and 1 soil biological. Treatments were tested *in vitro* using the contact method with the addition of Tween 80® at 1 %.

	Rate ppm	Tween 1%	*R. solani* (4 days)	*F. oxysporum* (7 days)	*P. dissotocum* (3 days)	*S.minor* (3 days)	*T.hamatum* (3 days)
Essential Oil	1	+	43.6 ± 5.6	49.9 ± 0.2	80.0	73.8 ± 6.2	80.0
Origanum	10	+	55.8 ± 0.5	54.6 ± 0.8	80.0	80.0	78.0 ± 2.0
	100	+	15.5 ± 1.0	24.2 ± 0.9	7.5 ± 0.3	62.9 ± 1.5	26.1 ± 1.9
	500	+	0.0*	0.0	0.0	0.0*	0.0
	500	-	0.0*	0.0	0.0	0.0*	0.0
Lemon myrtle	1	+	61.6 ± 5.9	56.6 ± 0.8	80.0	80.0	80.0
	10	+	59.7 ± 9.2	55.7 ± 0.6	80.0	80.0	80.0
	100	+	51.4 ± 1.0	50.1 ± 0.9	60 ± 20.0	80.0	80.0
	500	+	4.1 ± 2.8	24.8 ± 2.9	0.0	53.6 ± 6.0	14.8 ± 4.8
	500	-	0.0	4.2 ± 1.4	0.0*	0.0*	8.9 ± 2.0
Compound	1	+	50.3 ± 4.7	54.6 ± 0.5	39.6 ± 12.1	80.0	47.1 ± 1.5
Carvacrol	10	+	22.6 ± 3.2	46.5 ± 2.7	60.8 ± 2.0	80.0	48.7 ± 1.2
	100	+	0.0	3.8 ± 2.3	0.0	49.1 ± 2.8	12.7 ± 0.8
	500	+	0.0*	0.0*	0.0*	0.0*	0.0
	500	-	0.0*	0.0	0.0*	0.0*	0.0
Eugenol	1	+	52.5 ± 3.0	47.0 ± 0.5	80.0	80.0	80.0
	10	+	43.0 ± 5.6	54.4 ± 0.6	80.0	80.0	80.0
	100	+	26.7 ± 4.0	39.5 ± 1.0	15.1 ± 1.1	67.5 ± 2.5	74.0 ± 3.5
	500	+	0.0*	0.0	0.0	3.4 ± 3.4	2.5 ± 1.5
	500	-	0.0*	0.0	0.0	2.2 ± 2.2	1.0 ± 1.0
Product	1	+	58.6 ± 6.7	61.6 ± 1.6	80.0	80.0	80.0
Vigor®	10	+	63.4 ± 7.7	61.0 ± 0.6	80.0	80.0	80.0
	100	+	71.8 ± 0.3	55.8 ± 0.5	80.0	80.0	80.0
	500	+	52.8 ± 8.1	60.0 ± 0.7	80.0	80.0	80.0
	500	-	64.2 ± 1.2	59.5 ± 0.5	80.0	80.0	80.0
Untreated control	0	+	**71.8 ± 0.7**	**59.3 ± 1.7**	**80.00**	**80.00**	**80.00**

Mycelial colony diameter (mm) +/- SEM. 0= no growth observed, 80= colony grown to edge of plate
* biocidal activity (no mycelial growth observed after plugs were transferred to fresh PDA media)

5.3.2 Discussion

Preliminary *in vitro* screening of a broad range of plant volatile treatments identified six treatments which have strong antimicrobial activity. Thyme, clove bud and origanum showed inhibitory and biocidal activity against *F. oxysporum, R. solani, P. irregulare, P. aphanidermatum, P. sulcatum, P. dissotocum* (complex) and *S. minor.* These findings confirm previous studies which have demonstrated strong antimicrobial effects of thyme, oregano and clove against various important plant pathogens (Wilson *et al.* 1997; Montes-Belmont & Prados-Ligero, 2006; Tullio *et al.* 2007; Soylu *et al.* 2006, 2007; Lee *et al.* 2007). The antimicrobial activity of thyme, clove bud and origanum is likely due to their major chemical constituents thymol, eugenol and carvacrol respectively, as these compounds also showed strong antimicrobial activity against the test pathogens. These findings concur with previous studies (Muller-Riebau *et al.* 1995; Dorman & Deans 2000; Letessier *et al.* 2001).

The *in vitro* results showed obvious differences in antimicrobial potency between the contact and vapour bioassays, which have been reported widely in the literature. The majority of studies have found volatile effects of essential oils to be greater on pathogen growth than contact effect (Letessier *et al.* 2001; Tullio *et al.* 2007; Soylu *et al.* 2006). However the current study found that antimicrobial activity in contact phase was more potent than the vapour phase and showed a clear positive dose-dependant interaction. The inhibitory and biocidal effects of the plant volatiles in vapour phase were notably less potent and a dose-interaction was not consistent.

The volatile nature of plant volatiles means the vapours can evaporate over time thus losing their inhibitory effects. In a closed system such as a sealed perti dish, aromatic components will evaporate from the filter paper dish until the air space is saturated and equilibrium exists. Essential oils with higher volatility will therefore have a higher vapour concentration in the petri dish. The low volatility of eugenol has been suggested as the cause of its negative antimicrobial behaviour in vapour phase (Edris and Farrag 2003). Therefore the differences in volatility of plant volatile treatments may explain some of the variance of results of the vapour phase in this study. Furthermore it was proven difficult to place small amounts of pure product onto filter paper discs as well as keep the sterile discs in place, which also could have increased the variance of the results.

Thyme, clove bud, origanum and compounds thymol, eugenol and carvacrol showed remarkable antimicrobial effects against the test pathogens at relatively low concentrations compared to commercial plant volatile blends ECO-V® and Vigor®, which had much lower antimicrobial activity. Similarly Muller-Riebau *et al.* (1995) found that thymol, carvacrol and thyme oil inhibited growth of various tested organisms, and their efficacy against *Phytophthora capsicii* was greater than that of commercial fungicides. The ability of these treatments to inhibit mycelial growth as well as kill pathogen mycelium (biocidal activity) highlights their potential both as protectants at very low concentrations and as biofumigants at higher concentrations against soilborne pathogens of vegetables.

This study demonstrated broad spectrum antimicrobial activity of several plant volatile treatments, suggesting that differing modes of action may be causing antimicrobial affects against pathogen mycelium. A major benefit of multiple modes of action is that the development of pathogen resistance is less likely to occur because the pathogen has more than one mechanism to overcome. Since resistance development is one of the major issues with the use of synthetic chemicals for control of vegetable diseases, an added bonus of complex structures such as essential oils is that they are unlikely to lead to the development of pathogen resistance and may offer a more sustainable control option. Compounds with broad-spectrum protectant and biofumigant effects would be expected to provide protection against a range of pathogenic organisms and would be advantageous in controlling of disease complexes, whereby vegetable hosts may be affected by more than one pathogen at a time.

These findings confirm that several plant volatile treatments have strong antimicrobial effects and therefore should be investigated further as potential novel control options against vegetable diseases caused by soilborne pathogens *Fusarium, Pythium, Rhizoctonia* and *Sclerotinia* spp. in Australia. Our data on the antifungal properties of thyme, clove bud and origanum essential oils and their chemical compounds thymol, eugenol and carvacrol suggest these plant volatiles should be examined further to evaluate their potential as natural soil fumigants for disease control in vegetable production.

5.3 Pot bioassays to determine the disease control potential of selected plant-derived compounds

5.3.1 Materials and Methods

Three pot soil bioassays were conducted to evaluate the efficacy of plant-derived treatments for disease control. Treatments included the most effective essential oils identified in the *in vitro* bioassays (thyme, origanum and clove bud) as well as three plant volatile products (ECO-V®, Vigor® & Fumafert®) which were all compared to a commercial soil fumigant (Basamid®) for control of disease in pots. Wheat grain, sterilised twice at 121ºC for 60 mins, was inoculated with mycelial plugs from 7 day cultures of *R. solani* AG 2.1 (isolated from broccoli) and *P. irregulare* (isolated from parsley) and incubated at 21ºC for approximately 14 days. Mycelial covered wheat grain was blended with water into slurry and added to sterilised seed raising mix as inoculum at a rate of 25g/ kg. Inoculated seed raising mix was transferred into sealable plastic bags and treatments were added at various rates as either aqueous emulsions or solid amendments (Table 5.9). Un-inoculated wheat grain was added as slurry to control pots. All treatments received an equal volume of water. Bags were sealed, mixed thoroughly and left to fumigate for 48 hours. After fumigation, seed raising mix was dispersed into plastic 90 mm pots and placed in a random block design in the glasshouse. Five or six replicate pots of each treatment were used in each trial. After a further 7 days of incubation, all pots were planted with 5 seeds of either broccoli (*R. solani* bioassay) or pea (*P. irregulare* bioassay) and irrigated twice daily for 1 minute using overhead sprayers. Seedling emergence and disease incidence (broccoli) were assessed at 1 and 2 weeks after planting. Seedling emergence and fresh weights of peas were assessed at 1 week after planting.

Table 5.9 Treatments used in 3 bioassays for the evaluation of plant volatiles for control of soilborne diseases

Treatment	Mode of action	Application rate
Negative control (no treat)	-	n/a
Negative control (wheat control)	-	n/a
Positive control (inoculated, untreated)	-	n/a
Basamid® (chemical control)	MITC generation (fumigant)	200 kg/ha and 400 kg/ha
ECO-V® (mustard oil)	ITC volatiles	20 L/ha and 40 L/ha
Vigor® (Dazitol)	Allyl-ITC, capsaicin and other capsaicinoid volatiles	14 L/ha and 28 L/ha (diluted 15 % vigour conc:85 % water)
Fumafert®	ITCs (from mustard & neem)	1000 and 2000 kg/ha
Volatile plant essential oils	Biocidal plant volatiles	1, 5 and 10 % aqueous emulsion. (equivalent to 5.0 ml/150cm^3 soil)

5.3.2 Results and Discussion

The effect of plant-derived treatments on pea seedlings in *P. irregulare* inoculated pots

There was no difference in the mean emergence of pea seedlings between inoculated and un-inoculated controls (Table 5.10). No treatments increased seedling emergence, but pots treated with clove bud (5 & 10 %), origanum (5 & 10 %), thyme (10 %) and ECO-V® (low rate) reduced seedling emergence of peas compared to the untreated control (Table 5.10). The mean fresh weight of pea seedlings was highest in soil from the un-inoculated controls (Table 5.10). In inoculated soil, Basamid (low rate) and thyme (1 %) increased pea fresh weight by 60 % compared to the inoculated control (Table 5.10). Four treatments (origanum 1 & 10 %, thyme 10 % and fumafert® at the high rate) reduced (P<0.001) the fresh weight of pea seedlings compared to the inoculated control (Table 5.10).

Table 5.10 Summary of pea seedling emergence and seedling fresh weight in *P. irregulare* inoculated pots at 7 days after sowing

Treatment		Rate	Mean no. seedlings emerged	Emergence (%) compared to uninoculated control	Mean fresh weight (g)	% Fresh weight compared to inoculated control
Essential oil	Clove bud	1 %	4.0	80.0	23.1	28.5
		5 %	3.0	60.0	21.2	18.0
		10 %	3.2	64.0	19.6	9.0
	Thyme	1 %	4.2	84.0	28.6	59.3
		5 %	4.6	92.0	23.2	28.8
		10 %	0.8	16.0	8.2	-45.6
	Origanum	1 %	4.4	88.0	3.4	-18.7
		5 %	3.0	60.0	14.5	-80.8
		10 %	1.4	28.0	8.9	-49.6
Plant volatile	Fumafert	low	4.2	84.0	18.3	1.7
		high	4.0	80.0	9.3	-51.5
	Vigor®	low	4.0	80.0	16.1	-89.4
		high	4.2	84.0	23.9	32.8
	ECO-V®	low	3.4	68.0	18.7	3.9
		high	4.2	84.0	23.6	31.1
Chemical	Basamid®	low	4.8	96.0	28.5	58.5
		high	4.8	96.0	22.2	23.5
Control (uninoculated)		-	5.0	-	32.7	82.0
Wheat control (uninoculated)		-	4.4	88.0	51.4	185.9
Inoculated control		-	4.6	92.0	18.0	-
F-test			<0.001		<0.001	
lsd (P=0.001)			1.08		8.43	

The effect of plant-derived treatments on brassica seedling emergence in *R. solani* inoculated pots

Two bioassays were conducted to investigate the effect of plant-derived treatments on broccoli seedling emergence and seedling hypocotyl pruning caused by *R. solani*. The first bioassay investigated the effect of treatments on seedling emergence in *R. solani* inoculated soil only. The second bioassay investigated the effect of treatments on seedling emergence in *R. solani* inoculated soil as well as the effect of treatments on emergence in non-inoculated soil to identify potentially phytotoxic effects of the treatments on broccoli seedlings.

Results from bioassay 1 and 2 are presented in Tables 5.11 and 5.12 respectively. In bioassay 1, the mean number of broccoli seedlings emerged was highest in soil treated with Basamid® (highest rate), thyme 10 %, origanum 5 % and un-inoculated controls at 7 and 14 days after sowing. These treatments had significantly ($P<0.001$) higher numbers of broccoli seedlings germinated than the other treatments, all of which had less than 1 plant emerged including the inoculated control (Table 5.11). In bioassay 2 (7 days after sowing) *R. solani* inoculated pots treated with Basamid® (high rate), clove bud (5 %), fumafert, thyme (5 %) and ECO-V (high rate) had increased ($P<0.001$) seedling emergence compared to the untreated pots, which had 13 % seedling emergence (Table 5.12). At 14 days after sowing, Basamid® (high rate), clove bud (5 %), fumafert, thyme (5 %) and origanum (5 %) increased seedling emergence compared to the untreated control which had no seedlings emerged. The seedlings which had germinated in the inoculated control at 7 days had died by 14 days. This explains the reduction in seedling emergence falling from 13 % to 0 % (0.33 %) from 7 to 14 days after sowing.

These results were similar to Bowers and Locke (2000) who found treatment of soil with 5 % and 10 % aqueous emulsions of plant extract reduced pathogen population density and suppressed disease development of *Fusarium oxysporum* f. sp on musk melon. In the current study, some essential oil treatments were more effective than two commercial plant volatile blends Vigor® and ECO-V® as well as the fumigant Basamid® at a low rate, which indicates these treatments warrant further investigation as potential control options against vegetable diseases.

Some concentrations of origanum (5 % and 10 %) and clove bud (5 %) caused stunted broccoli seedlings, likely due to phytotoxicity. Basamid® and thyme (5 % and 10 %) were the most effective treatments in all of the experiments and did not show obvious phytotoxic effects. In other studies, essential oils were not found to have phytotoxic effects *in vivo* (Christan and Goggi 2008; Bajpai and Kang 2010)**.** In future experiments, the use of transplant seedlings may overcome issues of phytotoxicity due to mature plants being be more resilient than germinating seedlings, such as those used in these bioassays. These pot bioassays clearly show that thyme, clove bud and origanum oils have potential for plant disease control. Further work should be done to determine optimum rates and timing of application which minimise phytotoxic effects while still maintaining an acceptable level of disease control.

Table 5.11. Summary of results from pot bioassay 1 showing mean number of seedlings emerged and percent emergence of broccoli seedlings in *R. solani* inoculated soil at 7 and 14 days after sowing

			7 days		14 days	
Treatment		**Rate**	**Mean no. seedlings emerged**	**% emergence**	**Mean no. seedlings emerged**	**% emergence**
Essential oil	Clove bud	1 %	0.0	0.0	0.0	0.0
		5 %	0.0	0.0	0.0	0.0
		10 %	0.6	12.0	0.4	8.0
	Thyme	1 %	0.2	4.0	0.0	0.0
		5 %	0.0	0.0	0.2	4.0
		10 %	3.8	76.0	2.8	56.0
	Origanum	1 %	0.2	4.0	0.2	4.0
		5 %	3.6	72.0	1.6	32.0
		10 %	1.0	20.0	0.6	12.0
Product	Fumafert®	low	0.0	0.0	0.0	0.0
		high	0.0	0.0	0.0	0.0
	Vigor®	low	1.0	20.0	0.0	0.0
		high	0.2	4.0	0.2	4.0
	ECO-V®	low	0.2	4.0	0.2	4.0
		high	0.0	0.0	0.0	0.0
Fumigant	Basamid®	low	0.0	0.0	0.0	0.0
		high	5.0	100.0	5.0	100.0
Control (uninoculated)		-	4.8	96.0	4.8	96.0
Wheat control (uninoculated)		-	5.0	100.0	4.8	96.0
Inoculated control		-	0.2	4.0	0.2	4.0
F-test			<0.001		<0.001	
lsd (P=0.001)			0.987		1.0207	

Table 5.12. Summary of results from pot bioassay 2 showing the mean number of broccoli seedlings that germinated in non-inoculated and *R. solani* inoculated pots at 7 and 14 days after sowing

Treatment				7 days		14 days	
	Treatment	Pathogen	Rate	Mean seedling emergence	Total (%)	Mean seedling emergence	Total (%)
Control (uninoculated)	-	-	0	5.0	100	5	100
					87		
Wheat control (uninoculated)	-	-	0	4.3		4	83
Untreated control	-	+	0	0.7	13	0	0
Basamid®	+	+	200 kg/ha	0.5	10	1	40
	+	-	200 kg/ha	5.0	100	5	100
	+	+	400 kg/ha	4.2	83	5	40
	+	-	400 kg/ha	5.0	100	5	100
ECO-V (mustard oil)	+	+	20 L/ha	0.0	0	0	0
	+	-	20 L/ha	4.5	90	5	60
	+	+	40 L/ha	2.3	47	1	0
	+	-	40 L/ha	5.0	100	5	100
Vigor® (mustard oil)	+	+	14 L/ha	1.0	20	0	0
	+	-	14 L/ha	5.0	100	5	100
	+	+	28 L/ha	0.2	3	0	0
	+	-	28 L/ha	5.0	100	5	100
Fumafert®	+	+	1000 kg/ha	4.8	97	5	100
	+	-	1000 kg/ha	4.3	87	4	100
Thyme	+	+	5%	3.2	63	4	100
	+	-	5%	4.8	97	5	100
	+	+	10%	0.2	3	0	0
	+	-	10%	4.2	83	5	80
Origanum	+	+	1%	0.5	10	0	0
	+	-	1%	4.5	90	5	80
	+	+	5%	1.2	23	3	100
	+	-	5%	4.3	87	5	80
	+	+	10%	0.0	0	1	20
	+	-	10%	0.0	0	0	0
Clove bud	+	+	5%	3.8	77	4	40
	+	-	5%	2.3	47	4	100
	+	+	10%	0.0	0	0	0
	+	-	10%	0.5	10	1	0
treatment	F- test			<0.001		<0.001	
treat.pathogen				<0.001		<0.001	
treatment	Lsd			0.855		0.807	
treat.pathogen				0.988		0.9319	

5.4 Field studies to determine the disease control potential of selected plant-derived compounds

5.4.1 Materials and Methods

Experimental field sites

Field trials were conducted at two sites within different vegetable production regions to evaluate the potential of plant volatiles as a disease management option. The first site was established in a bean cropping system at Lindenow in Victoria (silty loam) which has a history of white mould (*Sclerotinia* spp.) and root rot (*Pythium, Fusarium* and *Rhizoctonia* spp.) diseases. The second site was established in a lettuce/leafy Asian vegetable cropping system at Heatherton in Victoria (sandy soil) which has a history of *Sclerotinia minor* and clubroot diseases.

Treatments and application

Seven treatments including six soil amendments and one foliar treatment were evaluated for disease control in commercial vegetable crops (Table 5.13). Commercial blends containing the essential oils previously evaluated were sourced and used for field trials at the highest recommended label rate. Treatments included commercial plant volatiles (Vigor®, ECO-V®, promax® (thyme), Bioweed™ and Fumafert®), a soil fertiliser (Perlka®), a soil fumigant (Basamid®) and a foliar fungicide for control of *Sclerotinia* (Filan®). With the exception of Filan® all treatments were applied as broadcast or drench onto cultivated soil three weeks before planting (Table 5.13). Filan® was applied as a foliar spray twice during flowering at 1 kg product/ha in 300L water with 60 ml Agral. The first spray was applied at 20-30 % flowering. The second spray was applied seven days later at full flowering.

At Lindenow treatments were applied to 12 x 3m plots. There were six replications of each treatment. Immediately after application treatments were incorporated by rotary hoe and were sealed with a roller to contain the volatiles. At Heatherton, treatments were applied to 9 x 1.5 m hilled plots. There were five replications of each treatment. After application the treatments were incorporated using a rotary hoe and were irrigated before being formed into raised beds. Three weeks after application the Lindenow site was sown to green beans (variety Valentino) and the Heatherton site was sown to lettuce (variety Green Butter). This site was subsequently sown to a leafy Asian vegetable (baby pak choi) after the lettuce crop had been harvested.

Table 5.13. Plant-derived treatments evaluated at the two field sites for their ability to control endemic soilborne pathogens at each site

Treatment	Rate[1]	Application method	Source
1. Untreated	-	-	-
2. Basamid® (dazomet) – chemical fumigant	500 kg/ha	Broadcast and incorporated	R&R Fumigation
3. ECO-V - biofumigant	50 L/ha[1]	Drench and incorporated	Prem Akhil
4. Vigor® (Dazitol) - biofumigant	50L/ha[1]	Drench and incorporated	Frencharoma c/o R&R Fumigation
5. Filan® (boscalid) - fungicide	1 kg/ha in 300L water with 60mL/100L Agral	Foliar (flowering) 2 sprays	NuFarm
6. Perlka® (calcium cyanamide) - fertiliser	1 t/ha	Broadcast and incorporated	Australian Agricultural Marketing Organisation
7. Promax™ (Thyme oil)	2 gallons/ac[1] (ie. 18.95 L/ha)	Drench and incorporated	Bio HumaNetics
8. Bioweed™ (Pine oil)	90 L/ha[1]	Drench and incorporated	Certified organics
9. Fumafert® (mustard meal & neem cake) - organic fertiliser	2 t/ha	Broadcast and incorporated	Organic crop protectants

[1] All pre-planting liquid treatments were drenched over plots in 27L water per plot at Lindenow and 9 L water at Heatherton.

Assessments and statistical analysis

At the Lindenow site, bean crop yield and disease were assessed at harvest. Whole plants were removed in 1m² quadrats in the middle two rows of each plot. Bean plants were assessed for root rot severity (scale of 0-5), incidence of root rot (%) and white rot (number and weight of infected and healthy pods) and biomass (total number and weight of plants). Total and average fresh weights and root rot incidence and severity were analysed using analysis of variance (Genstat 12th edition). At the Heatherton site, the cumulative number of infected lettuce plants was recorded at 2-week intervals until harvest. The second crop pak choi was assessed six weeks after sowing for yield (total plant weight per plot) and clubroot disease incidence and severity (0-9). Total fresh weight and clubroot disease scores were analysed using analysis of variance.

Soils were tested for levels of pathogen inoculum (soil DNA) using the South Australian Research and Development Institute (SARDI) soil testing service. The assays use a TaqMan® real-time quantitative PCR (Polymerase Chain Reaction) assay (Ophel-Keller, K. *et al*. 2008) to provide a quantitative measure of pathogen inoculum in soils (*Pythium* clade f*. and *Rhizoctonia solani,* AG2-1). Soil samples were collected in a W formation (following the SARDI sampling protocol) immediately before planting. Total soil DNA was analysed using analysis of variance. Data were transformed using square root and log transformations when required before analysis and when variance indicated a significant treatment effect, fisher's LSD tests (5 % level) were used to compare treatment means.

* *Pythium* clade f refers to a group of *Pythium* species descended from one common ancestor. It may contain members that are pathogenic and non-pathogenic. Routine detection of *Pythium* spp. from soil is currently available for clades, not individual species.

5.4.2 Results

The effect of selected plant-derived treatments for control of Sclerotinia white mould and root rot diseases of green beans in a commercial crop – Lindenow Victoria

Basamid®, ECO-V® and Vigor® treatments significantly reduced the severity of root rot symptoms (Table 5.14) compared to the untreated plots but only Basamid® also significantly reduced root rot incidence. There was no effect (P>0.05) of the treatments on total fresh weight or average plant weight of green beans (Table 5.14).

There was insufficient white mould disease (*Sclerotinia* spp.) in the beans at this site to report treatment effects (data not included).

Table 5.14. Effect of soil treatments and commercial products on plant weights and root rots caused by soilborne diseases on green beans

	Plant weights		Root rot		
Treatment	Total fresh weight (kg)	Average plant weight (g)	Number of plants infected	% incidence	Average severity
Perlka®	1.81	85.10	21.50b	100.00b	4.00b
Fumafert®	1.69	85.10	20.00b	99.17b	3.92b
Filan®	1.39	62.90	21.26b	100.00b	3.58b
Bioweed™	1.61	70.10	22.50b	100.00b	3.75b
Promax™	1.64	73.80	22.50b	100.00b	3.42b
Vigor®	1.50	63.10	23.67b	100.00b	3.33a
ECO-V®	1.83	82.20	22.50b	98.33b	3.25a
Basamid®	1.66	80.80	17.33a	80.00a	2.92a
Untreated	1.79	77.30	23.50b	100.00b	4.00b
F-test	0.492	0.072	0.065	<0.001	0.024
lsd (P=0.001)	0.492	17.59	3.938	8.054	0.672

The effect of selected plant-derived treatments for control of lettuce drop (Sclerotinia) in lettuce and clubroot of pak choi in commercial vegetable production – Heatherton Victoria

With the exception of Basamid® (a fumigant) and Filan® (a fungicide) none of the treatments significantly (P≤0.05) reduced the incidence of lettuce drop caused by *Sclerotinia* spp. Likewise only Basamid® significantly reduced the severity of symptoms of clubroot disease in the subsequent baby pak choi crop at the Heatherton site. This treatment was also the only treatment which increased (*P*=0.045) baby pak choi fresh weight (Table 5.15).

Table 5.15. Effects of plant-derived and commercial treatments on pak choi fresh weight and clubroot severity and incidence of sclerotinia in lettuce in commercial site in Heatherton, Victoria (2009-2010)

	Pak choi		Lettuce	
Treatment	**Mean fresh weight (g)**	**Club root score (0-9)**	**Total no. infected (lettuce drop)**	**% infection compared to control**
Basamid®	1.0a	5.8a	6.0a	53.3
Filan®	-	-	4.3a	37.8
Perlka®	0.7b	8.6b	11.5b	102.2
Bioweed™	0.7b	7.9b	13.3b	117.8
Promax™	0.7b	7.8b	14.0b	124.4
Vigor®	0.5b	8.5b	13.0b	115.6
ECO-V®	0.5b	8.5b	13.0b	115.6
Untreated	0.7b	8.2b	11.3b	-
F-test	0.045	<0.001	<0.001	-
lsd (P=0.001)	0.2723	0.957	4.429	-

The effect of plant-derived treatments and commercial products on pathogen soil DNA in field experiments

At the Lindenow field site, Basamid® significantly reduced *R. solani* soil inoculum while Fumafert® and Perlka® were promising treatments against this pathogen. No treatments had a significant effect on soil inoculum of *Pythium* clade f. at Lindenow (Table 5.16). At the Heatherton field site, Basamid® significantly reduced *Pythium* clade. f soil inoculum while Bioweed™ and Perlka® were promising treatments (Table 5.17). Basamid®, Bioweed™, Promax®, ECO-V® and Fumafert® also showed promise against *R. solani* at Heatherton but the amount of soil inoculum was too low at this site for reliable conclusions to be drawn from the data (Table 5.17).

Table 5.16. Effects of treatments soil inoculum in a field trial in Lindenow, Victoria

Treatment	*R. solani* pgDNA/g soil	*Pythium* clade f. pgDNA/g soil
Perlka®	983 b	217
Fumafert®	645 b	339
Bioweed™	1188 b	258
Promax™	1051 b	271
Vigor®	1307 b	239
ECO-V®	1054 b	248
Basamid®	390 a	214
Untreated	1307 b	285
F-test	0.377	0.698

1 Data was transformed as required; values within a column with different letters are significantly different

Table 5.17. Effects of treatments soil inoculum in a field trial in Heatherton, Victoria.

Treatment	*R. solani* pgDNA/g soil	*Pythium* clade f. pgDNA/g soil
Perlka®	31.7	50.5 b
Fumafert®	13.0	148.2 b
Bioweed™™	5.7	46.9 b
Promax	8.9	157.6 b
Vigor®	34.2	89.1 b
ECO-V®	12.1	83.4 b
Basamid®	0.0	3.6 a
Untreated	73.2	107.9 b
F-test	0.363	0.119

1 Data was transformed as required; values within a column with different letters are significantly different

5.4.3 Discussion

There are limited studies that have investigated the efficacy of plant volatiles for control of vegetable diseases in the field which makes it difficult to compare and confirm results. Results from both field trials in this study have indicated that the soil treatments tested did not provide adequate control of soilborne diseases compared to the soil fumigant basamid under moderate to high disease pressure. However Vigor® and ECO-V® were promising treatments against root rot of beans at one field site. The lack of efficacy of some treatments in the field may be due to the application method used in these trials. A study by Ji *et al.* (2005) showed that thymol (active compound in thyme oil) significantly reduced incidence of bacterial wilt of tomato caused by *Ralstonia solanacearum* applied as 200 ml aqueous emulsion (thymol 1.4g, 70 % ethanol 7 ml, water 191.6 ml, detergent 200 µl) and sealed under polyethylene mulch. The technique used in field trials of this study which attempted to contain volatiles by creating a compact soil crust using a roller after application, may not adequately contain volatiles enough to have an effect. Due to the volatility of some plant volatile products, treatments may volatilise readily. Incorporation methods and irrigation regimes may be critical elements which could affect the coverage and therefore efficacy of soil treatments when targeting soilborne pathogens. The efficacy of volatile treatments may be improved by investigating formulation techniques to reduce volatility and prolong the activity and coverage in soil (Walter *et al*. 2001).
Previous studies by Walter *et al*. (2001) found thyme oil controlled bunch rot foliar infections of grapes and Ji *et al*. (2005) showed that thymol controlled bacterial wilt of tomato. The lack of efficacy of promax, a thyme oil based commercial product used in this study, may be attributed to the low concentration of thyme oil (3.5 %) in this product. As there were no phytotoxic effects observed on the crops at either field site and there were was relatively no affect of soil treatments on disease control, it is again likely that the rates used were not adequate to achieve control or that the application technique was not effective.

Studies have evaluated efficacy of plant volatiles as seed treatments *in vivo* and others have assessed their efficacy against disease as foliar sprays, yet few studies have tested them as soil treatments as this study has done. Based on other field trials and our findings in pot bioassays, thyme oil warrants further investigation at higher concentrations in the field against soilborne diseases of vegetables. Further studies are needed to clarify several issues such as dosage and method of application in field conditions (which will depend on specific cropping practices) to achieve maximum disease control efficacy and economic benefit.

5.5 Conclusions

Plant volatiles are a novel control measure being investigated against plant pathogens. These studies clearly show the inhibitory and biocidal activity of thyme, clove bud and origaum against soilborne pathogens of vegetables *in vitro*, however their efficacy *in vivo* were more difficult to determine. Thyme, clove bud and origanum clearly show potential for disease control in pot bioassay studies at rates of 5 % and 10 % aqueous emulsions. In some instances, these oils provided similar control to that of soil fumigant basamid. However these trials highlighted the issue of phytotoxicity, which have been found in previous studies (Walter *et al.* 2001). Further trials should be done to optimise application rates and exposure times, in order to provide adequate levels of control without causing phytotoxicity. Field trials were less conclusive. The soil fumigant Basamid® was more effective than all other treatments under moderate to high disease pressure. Results from this study indicate that the concentrations of the treatments used in the field trials are insufficient to protect the crop or that the volatile compounds diffused from the soil surface. Therefore increasing concentrations of treatments or altering the delivery method should be investigated future experiments, as well as investigations to address safety (all of the products had a strong odour and would be unsuitable for long term human exposure) and economic feasibility.

5.6 References

Bajpai, V.K. and Kang, S.C. (2010) Antimicrobial activity of leaf essential oil and extracts of *Metasequoia glytostroboides* Miki ex Hu. *Journal of the American Oil Chemists' Society* 87, 327-336.

Bowers, J.H. and Locke, J.C. (2000) Effect of botanical extract on the population density of *Fusaruim oxysporum* in soil and control of *Fusaruim* wilt in the greenhouse. *Plant Disease* **84,** 300-305.

Christian, E.J. and Goggi, A.S. (2008). Aromatic plant oils as fungicide for organic corn production. *Crop Science* 48, 1941- 1951.

Cowan, M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews* 12 (4), 564-582.

Dorman, H.J.D. and Deans, S.G. (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology* 88, 308-316.

Edris, A.E. and Farrag, E.S. (2003) Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapour phase. *Nahrung/ Food* 47 (2), 117-121.

Ji, P. Momol, M.T. Olson, S.M. and Pradhanang, P.M. (2005) Evaluation of thymol as biofumigant for control of bacterial wilt of tomato under field conditions. *Plant Disease* 89 (5), 497– 500.

Kalemba, D. and Kunicka, A. (2003) Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry* 10, 813-829.

Lee SO, Choi GJ, Jang KS, Lim HK, Kwang YC, Kim J (2007) Antifungal activity of five plant essential oils as fumigant against postharvest and soil-borne pathogenic fungi. *Plant Pathology Journal* **23** (2), 97-102.

Lee, Y. Kim, J. Lee, S. Oh, E. Shin, S. and Park, I. (2009) Effects of plant essential oils and components from oriental sweetgum (*Liquidambar orientalis*) on growth and morphogenesis of three phytopathogenic fungi. *Pesticide Biochemistry and Physiology* 93, 138- 143.

Letessier, M.P. Svoboda, K.P. and Walters, D.R. (2001) Antifungal activity of essential oil of hyssop (*Hyssopus officinalis*). *Journal of Phytopathology* 149, 673-678.

Montes- Belmont, R and Prados-Ligero, A.M. (2006) Influence of plant extracts on *Sclerotium cepivorum* development. *Plant Pathology Journal* 5 (3), 373- 377.

Muller-Riebau, F. Berger, B. and Yegen, O. (1995) Chemical composition and fungitoxic properties to phytopathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. *Journal of Agricultural and food chemistry* 43, 2262- 2266.

Ophel-Keller, K. McKay, A. Hartley, D. Herdina, Curran, J. (2008) Development of a routine DNA-based testing service for soilborne diseases in Australia. *Australasian Plant Pathology* 37, 243–253.

Pandey DK, Tripathi NN, Tripathi RD, Dixit SN (1982) Fungitoxic and phytotoxic properties of the essential oil *Caesulia axillaris* Roxb. *Angewan Botany* **56**, 259–267.

Soylu, E.M., Soylu, S. and Kurt, S. (2006) Antimicrobial activities of the essential oils of various plants against tomato late blight agent *Phytophthora infestans. Mycopathologia* 161, 119- 128.

Soylu, S., Yigibas, H., Soylu, E.M. and Kurt. S. (2007) Antifungal effects of essential oils from oregano and fennel on *Sclerotinia sclerotiorum. Journal of Applied Microbiology* 103, 1021-1030.

Tullio, V., Nostro, A., Mandras, N., Dugo, P., Banche, G., Cannatelli, M.A., Cuffini, A.M. (2007) Antifungal activity of essential oils against filamentous fungi determined by broth microdilution and vapour contact methods. *Journal of Applied Microbiology* 102, 1544-1550.

Walter, M. Jaspers, M.V. Eade, K. Frampton, C.M. and Stewart, A. (2001) Control of *Botrytis cinerea* in grape using thyme oil. *Australasian Plant Pathology* 30, 21-25.

Wilson, C.L., Solar, J.M., El Ghauth, A. and Wisniewski, M.E. (1997) Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea. Plant Disease* 81 (2), 204-210.

Zambonelli, A. Zechini D' Aulerio, A. Bianchi, A. and Albasini, A. (1996) Effects of essential oils on phytopathogenic fungi *In Vitro*. *Journal of Phytopathology* 144, 491-494.

Disruption of fungal resting structures

6. Disruption of fungal resting structures

Researcher: Alicia Greenhill (PhD candidate)
Supervisors: Kim Plummer La Trobe University and Ian Porter Department of Primary Industries Victoria

Melanised fungal structures called sclerotia or micro-sclerotia enable many fungal pathogens including *Sclerotinia* spp. to survive for long periods in soil even in the absence of a suitable host. The outer melanised layer of these structures is resistant to degradation and parasitism. Understanding the genetic control of sclerotial development may lead to the identification of targets for sustainable control of *Sclerotinia* and other soilborne pathogens. This fundamental research has identified the pathway by which *Sclerotinia* produces melanin. Chemical disruption of this pathway resulting in inhibition of sclerotial development and/or melanisation has been demonstrated. In summary the work has demonstrated that:

- *Sclerotinia* produces melanin via the DHN biosynthesis pathway in mycelia and sclerotia
- tricyclazole, and to a lesser extent, pyroquilon, affect sclerotial formation in *Sclerotinia*
- it is possible that *S. sclerotiorum* and *S. minor* also employ another melanin biosynthesis pathway, eg: L-DOPA
- sclerotia-deficient or aberrant mutants can be used to identify genes involved in sclerotial formation. The genes identified may provide targets for sustainable control of *Sclerotinia* and other soilborne plant pathogens

The work detailed in this chapter was conducted by Alicia Greenhill as part of a PhD project undertaken as part of the wider program.

6.1 Introduction

Many soilborne plant pathogens produce resting structures called sclerotia or micro-sclerotia (Table 6.1). These structures often have an outer layer that is highly melanised and resistant to degradation or parasitism which enables them to survive in soil for many years. In some cases, propagule loading via sclerotia can become so severe that infected fields are no longer viable for crop production (Zhou & Boland, 1998). High loading of sclerotia was highlighted as key cause of disease control failures at the *Sclerotinia* workshop conduced at the beginning of the project (Tasmania, 2007). Nutritional and environmental factors that influence sclerotial production have been described (Chet & Henis, 1975; Le Tourneau, 1979; Willets & Wong, 1980; Willets & Bullock, 1992), however the molecular mechanisms that trigger the morphological change from mycelial growth to sclerotial formation are yet to be identified.

← Image previous page, chapter 6 coversheet – sclerotia of *Sclerotinia sclerotiorum* with apothecia from which airborne ascospores will be released.

Table 6.1. Examples of some sclerotia producing organisms and diseases they cause

Organism	Examples of disease/hosts
Sclerotinia sclerotiorum	Sunflower wilt, cottony soft rot, white mould, lettuce drop (sunflower, canola, most vegetables, legumes, lettuce)
Sclerotinia minor	Lettuce drop, watery soft rot (lettuce, vegetables)
Sclerotium rolfsii	Southern blight (vegetables, fruit, ornamentals)
Botrytis cinerea	Bunch rot (grape), grey mould (strawberry)
Rhizoctonia solani	Damping off, wire stem (vegetables, cereals, ornamentals)
Verticillium dahliae	Verticillium wilt (potato, strawberry, herbs)

Sclerotinia sclerotiorum is a particularly prolific soil-borne plant pathogen. It is capable of infecting over 380 plant species (Purdy, 1979) including many economically important crop species. Oilseed crops including soybean, sunflower and canola, and vegetable crops such as lettuce, tomato and bean incur great loss due to *Sclerotinia* disease. The genome of *S. sclerotiorum* has been sequenced and is publicly available, making it an ideal model for studying the molecular mechanisms of sclerotial formation. Additionally, it is closely related to other plant pathogens such as *Sclerotinia minor* and *Botrytis cinerea,* which means that findings in *S. sclerotiorum* may be extrapolated and applied to these pathogens too.

Melanins are dark pigments found in a wide range of organisms including phytopathogenic fungi. Melanins can be found in the hyphae of such organisms (eg: as in *R. solani*) and also in the outer layer (rind) of resting structures such as sclerotia (eg: as in *S. sclerotiorum* (Figure 6.1) and *S. minor*). This melanisation directly contributes to long term survival of propagules in soil by protecting the sclerotia or hyphae against parasitism by soil micro-organisms and other environmental stresses (Bloomfield & Alexander, 1967; Kuo & Alexander, 1967; Huang, 1982; Huang, 1985). Naturally occurring variants of *S. sclerotiorum* that produce improperly melanised, tan-coloured sclerotia are more highly parasitised in soil, degrade faster, and are less viable than black sclerotia (Huang, 1983).

Inhibition of melanin biosynthesis could therefore lead to sclerotia that are less robust and viable than the black sclerotia normally produced by *Sclerotinia,* potentially reducing the amount of inoculum that remains in an infected field after an outbreak of *Sclerotinia* disease.

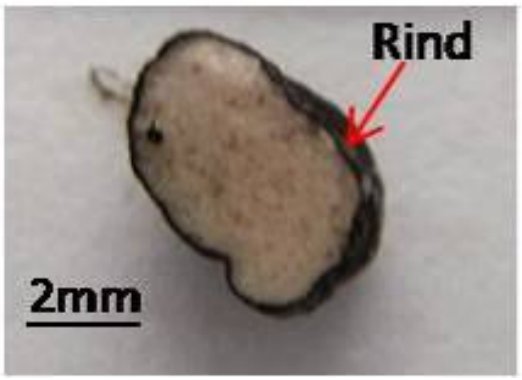


Figure 6.1. Photo of *S. sclerotiorum* sclerotium, from an *in vitro* culture, cut in half to expose the rind and the inner, creamy white medulla.

There are four melanin biosynthesis pathways reported in fungi – GHB (glutaminyl-4-hydroxybenzene) catechol, DHN (1,8 dihydroxynapthalene) and DOPA (Fleet & Breuil, 2002, Solomon *et al,* 2004). DHN melanin, also referred to as pentaketide melanin, has thus far been found to be the most prevalent melanin in phytopathogenic fungi (Butler & Day, 1998). A number of compounds are known to inhibit DHN melanin biosynthesis (Figure 6.2). Reductase melanin biosynthesis inhibitors (MBI-R's) inhibit DHN melanin synthesis at the steps indicated by an [H] in Figure 6.3, and dehydratase melanin biosynthesis inhibitors (MBI-Ds) inhibit at the $-H_2O$ steps (Figure 6.3).

It has been established that *S. sclerotiorum* produces melanin via the DHN melanin biosynthesis pathway by examining the phenotype of *S. sclerotiorum* cultures exposed to the potent compound tricyclazole (Butler *et al,* 2009). This work has been repeated in this project. Additionally, the DHN melanin biosynthesis pathway has been characterised and the genes encoding relevant enzymes have been identified in several other organisms (eg: Tsai *et al*, 1997; Thompson *et al*, 2000). Orthologs of these genes are present in the genome of *S. sclerotiorum,* presenting further evidence that *Sclerotinia* produces DHN melanin.
Therefore, the following investigations were conducted:

1. Testing for chemical inhibition of melanin biosynthesis in *S. sclerotiorum* and *S. minor*
2. Identifying when genes encoding melanin biosynthesis genes are 'switched on'
3. Disruption of these genes to determine how this effects sclerote formation

In an alternative approach, sclerotia aberrant mutants were studied to identify genes involved in the formation of sclerotia. T-DNA mutants of *S. sclerotiorum* have been created by the University of Florida via *Agrobacterium*-mediated transformation (AMT). In this process a short DNA sequence (T-DNA) is inserted randomly into the fungal genome (Fig 6.4). As the sequence of this t-DNA tag is known, the gene or region into which it has been inserted can be identified. A number of these mutants display a sclerotia-minus, or sclerotia-aberrant phenotype. These mutants were sent to La Trobe University for study as part of this project.

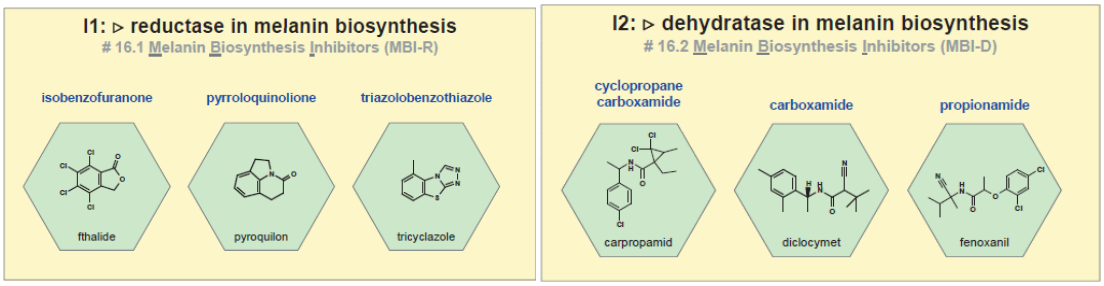


Figure 6.2. Chemical makeup of compounds that inhibit DHN melanin synthesis either at the reductase or dehydratase steps in the pathway (Figure from FRAC 2009 poster).

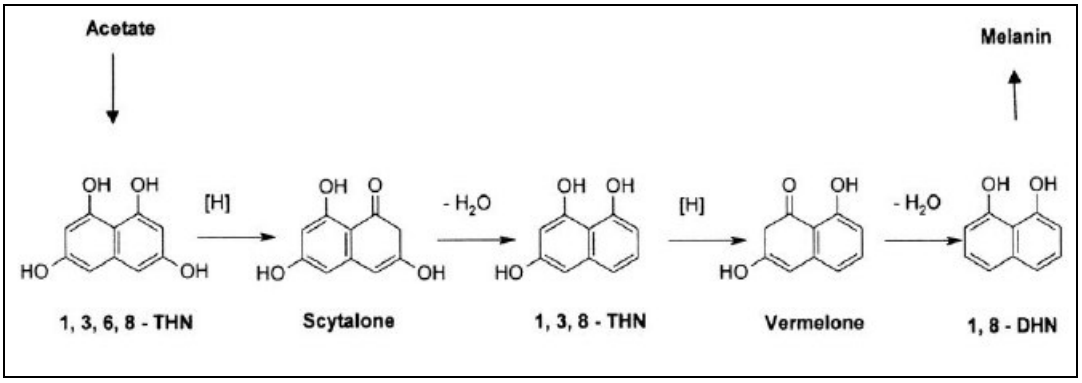


Figure 6.3. The DHN melanin biosynthesis pathway [H] refers to steps undertaken by hydroxynapthalene reductases. $-H_2O$ refers to steps undertaken by dehydratase enzymes. (Figure from Butler *et al,* 2005).

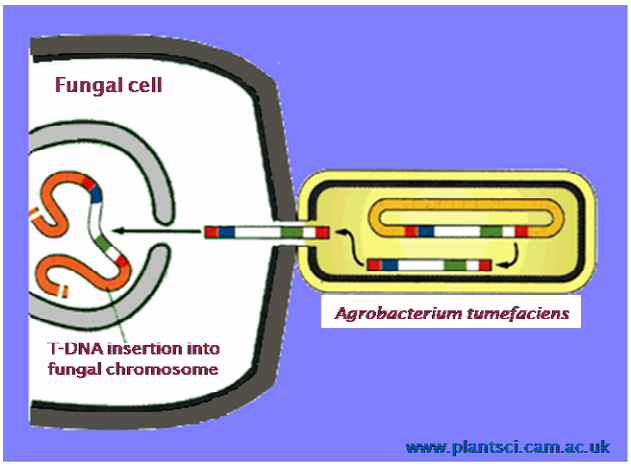


Figure 6.4 Diagram outlining *Agrobacterium*-mediated transformation of fungi. (Adapted from www.plantsci.cam.ac.uk)

6.2 The role of melanin in sclerotial formation

6.2.1 Chemical inhibition of melanin

Materials and methods

Trials of melanin inhibitors have been repeated 3 times with various adaptations. The following methods and results are from the second trial, with the exception of Figure 6.7 which comes from the third trial.

Study species/isolate

The isolates of *S. sclerotiorum* and *S. minor* tested in this trial were UQ1280 (University of QLD) and LaU (Field isolate, from lettuce on Lamattina's farm, upper field, Boneo, Victoria) respectively.

Inhibition of melanin in vitro

The DHN melanin inhibitors pyroquilon and tricyclazole were tested in this trial. Kojic acid, an L-DOPA melanin inhibitor, was also tested. Compounds were dissolved or suspended (tricyclazole) in ethanol and added to Potato Dextrose Agar (PDA) cooled to 55°C at the concentrations listed in Table 6.2. Approximately 10 ml of amended media was poured into small plastic dishes and left to set in sterile conditions under a laminar flow.

A single 5mm plug from the growing edge of a colony on PDA was placed mycelia side down onto the centre of each plate and the plates sealed with Parafilm. Four replicates of each treatment were included for each isolate, which were numbered and stacked into an open container left in the dark at room temperature.

Photos were taken at days 7 and 15 to record results of all four replicates. In addition to this a replicate that best represented the average phenotype of each treatment was photographed alongside the control and other treatments. Photos were taken with a Pentax Optio 6 megapixel camera.

Table 6.2. Concentrations of compounds added to PDA to test melanin inhibition in *Sclerotinia.* (PPM = parts per million)

Chemical/ control	Compound concentration
Negative control	-
100 % Ethanol	1 % volume:volume
Tricyclazole	50 & 100 PPM
Pyroquilon	50 & 100 PPM
Kojic acid	50 & 100 PPM
Tricyclazole + Kojic acid	50 PPM of each
Pyroquilon + Kojic acid	50 PPM of each

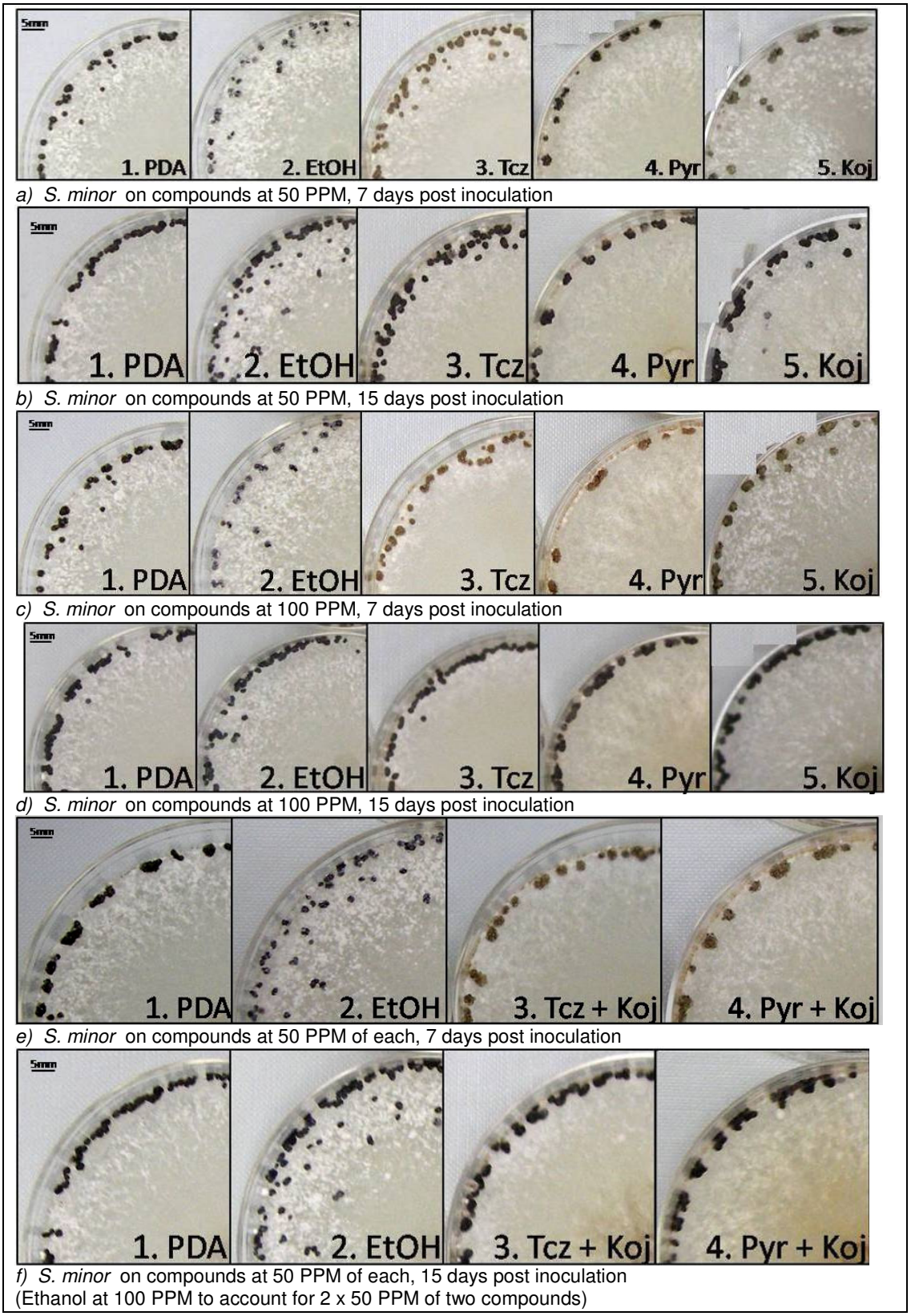
nb: for the results shown in Figure 6.8 compounds were dissolved in DMSO rather than ethanol and the *S. sclerotiorum* isolate 1980 (US isolate) was used.

Results and discussion

Growth, melanisation of mycelia and number of sclerotia in *S. sclerotiorum* produced on the control plates was quite variable between technical replicates (Figures 6.6, 6.7b & 6.8). This variation was echoed in the treatments, however plates which best represented the average phenotype of each treatment were selected to photograph. This variation was not as pronounced in *S. minor* plates which were much more uniform both in colouration and size/number of sclerotia produced (Figures 6.5 and 6.7a). Inclusion of ethanol in the media appeared to have a slight effect on the mycelia of *S. minor* (Fig. 6.5). However, there was no significant deviation in sclerotial colouring, size or proper rind and medulla formation in control plates of either solvent for either *S. minor* or *S. sclerotiorum* (Figs. 6.5-6.8).

Tricyclazole and pyroquilon both have an inhibitory effect on melanisation of mycelia, producing a red/tan colouring in both species (Figs. 6.5 & 6.6). This tan colour is caused by shunt products formed after inhibition of melanin biosynthesis (Butler *et al,* 2009) and indicates that both species produce DHN melanin. It is possible that melanised mycelia protects *Sclerotinia* against antagonistic microbes (Butler *et al,* 2009). Growth on tricyclazole impedes normal formation of sclerotia in *Sclerotinia.* In *S. minor,* growth on tricyclazole leads to abnormal melanisation of the sclerotial rind in early stages of sclerotial development (Fig 6.6a) at both 50 and 100PPM. However, after 15 days the sclerotial rind appears to have melanised normally in all treatments. At high concentrations tricyclazole slows down production of sclerotia in *S. sclerotiorum.* At 15 days only initials have been formed (Fig 6.7b & 6.8) and sclerotia are not matured until approximately day 28 (Fig 6.8). Pyroquilon and kojic acid also cause abnormal melanisation of sclerotia at day 7 at 100 PPM in *S. minor,* however normal melanisation has occurred in the mature sclerotia at day 15 (Fig 6.7a). Both compounds appear to have little effect on sclerotia in *S. sclerotiorum* other than a slight developmental retardation at day 7 that is not evident by day 15 (Fig 6.6 & 6.7b).

These early differences in sclerotial melanisation/development which are not evident in the mature sclerotia (at a macroscopic level) could be due to degradation of the chemicals, *Sclerotinia* overcoming the treatments, or the initiation of a secondary melanin biosynthesis pathway (eg: L-DOPA). If the other pathway is L-DOPA, growth on both kojic acid and tricyclazole/pyroquilon should show complete inhibition of melanin. At 50 PPM of each there is negligible difference from control plates (Fig 6.5,6.6 & 6.7). At 100 PPM (data not shown) phenotype is very similar to that of tricyclazole or pyroquilon alone. However, chemical inhibition cannot be relied upon solely as this sort of experiment sometimes gives a false result depending on the parameters tested (Butler *et al,* 2009). Therefore this is not enough evidence to conclusively rule out or confirm a secondary pathway in *Sclerotinia.* There is other potential evidence for L-DOPA melanin synthesis in *Sclerotinia.* Butler *et al.* (2009) report that DHN-melanin producers grown on low pH ascorbate media form albino cultures: *S. sclerotiorum* does not; suggesting a secondary pathway. Additionally, genes relevant to the L-DOPA synthesis pathway are encoded in the genome of *S. sclerotiorum.* Further experimentation is needed.



a) S. minor on compounds at 50 PPM, 7 days post inoculation

b) S. minor on compounds at 50 PPM, 15 days post inoculation

c) S. minor on compounds at 100 PPM, 7 days post inoculation

d) S. minor on compounds at 100 PPM, 15 days post inoculation

e) S. minor on compounds at 50 PPM of each, 7 days post inoculation

f) S. minor on compounds at 50 PPM of each, 15 days post inoculation
(Ethanol at 100 PPM to account for 2 x 50 PPM of two compounds)

Figure 6.5 Results from chemical trial of *S. minor* grown on various compounds *in vitro*

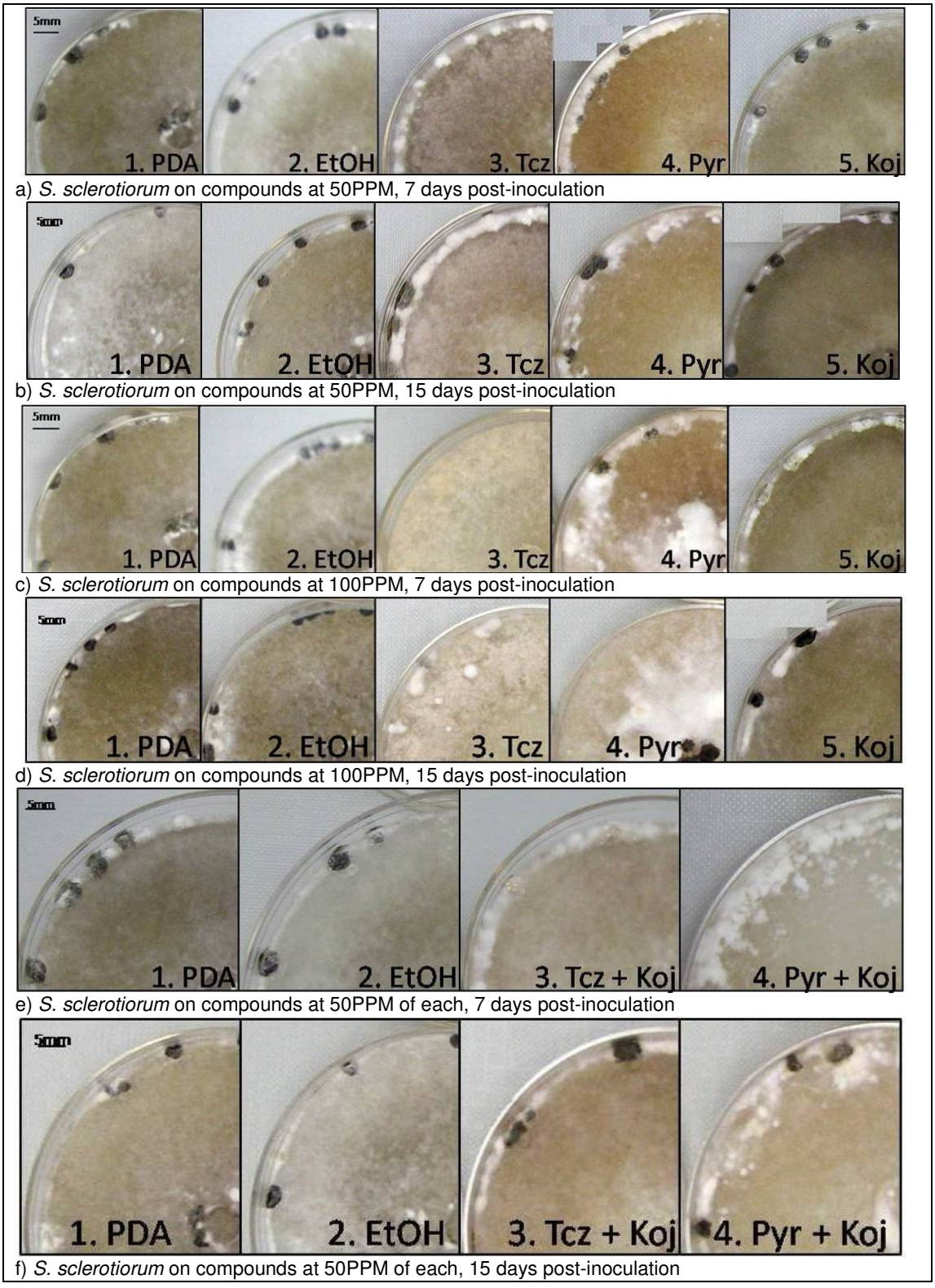


Figure 6.6 Results from chemical trial of *S. sclerotiorum* grown on various compounds *in vitro*

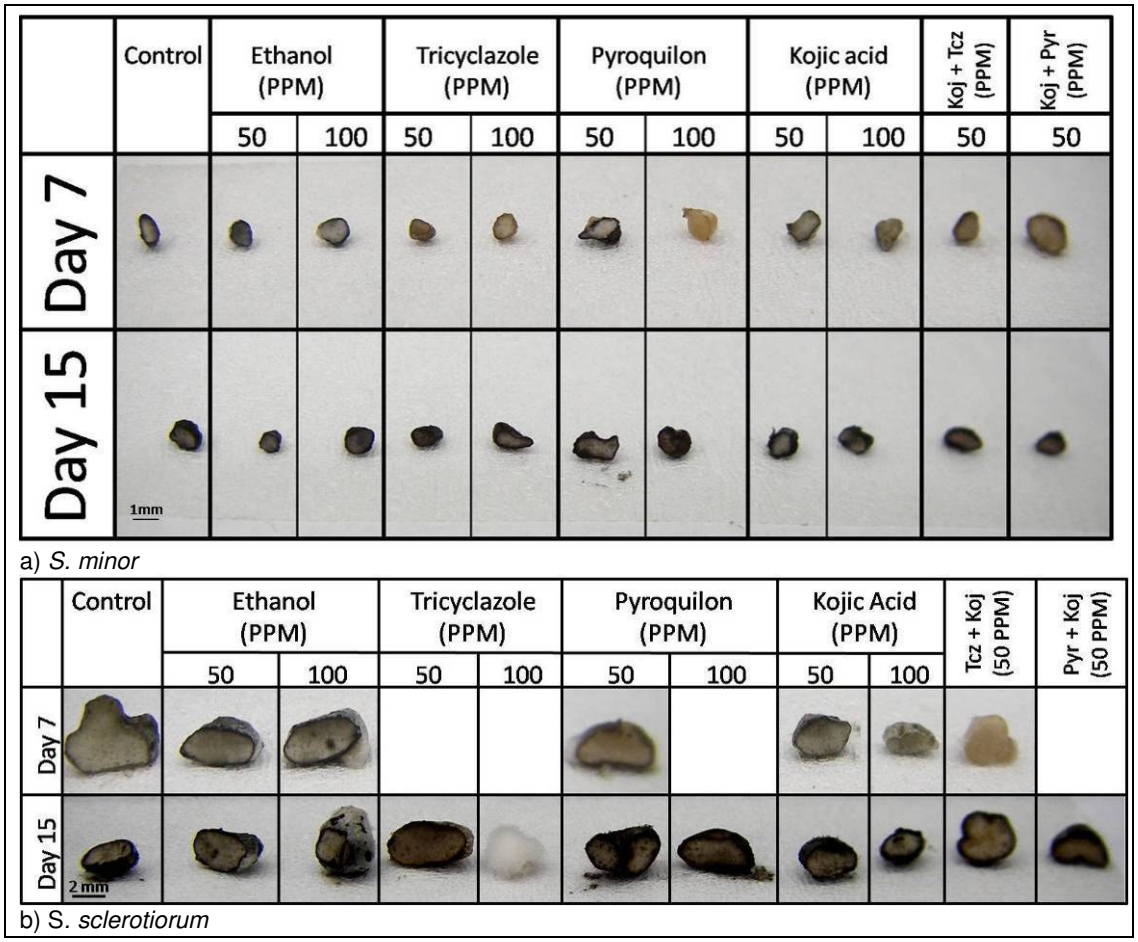


Figure 6.7 Cross sections of *S. minor* (a) and *S. sclerotiorum* (b) sclerotia taken from PDA treated with various compounds at 7 and 15 days post-inoculation.

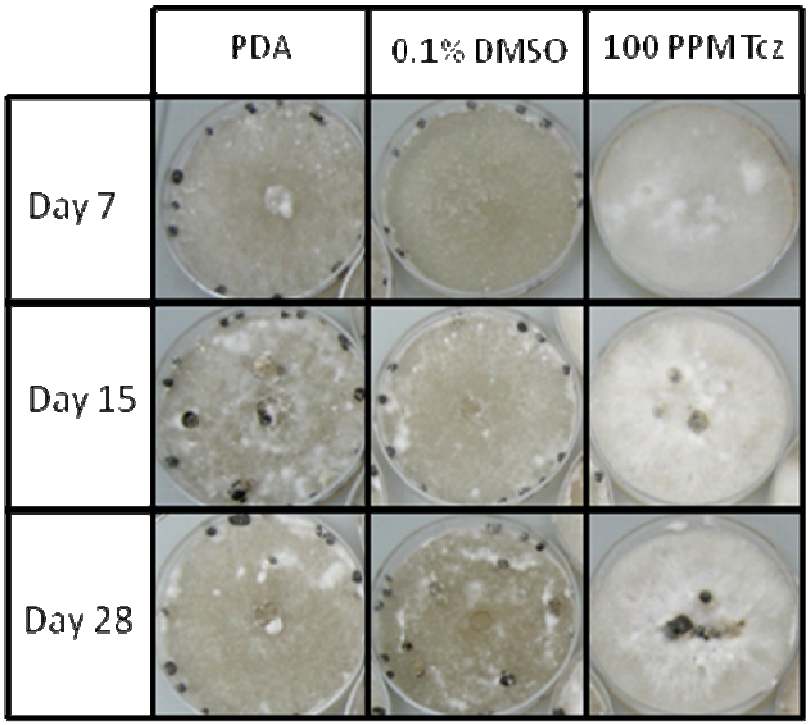


Figure 6.8 *S. sclerotiorum* grown on PDA, PDA + 0.1 % DMSO and 100PPM tricyclazole, photographed at 7, 15 and 28 days post inoculation (from trial 3).

6.2.2 Expression of melanin biosynthesis genes

Materials and methods

Genes encoding enzymes on the DHN melanin biosynthesis pathway have been identified and characterised in other organisms (eg: Tsai *et al*, 1997; Thompson *et al*, 2000). Orthologs of these genes are present in the genome of *S. sclerotiorum*, but there is no published evidence that these genes are expressed. Two genes (Scytalone dehydratase: Ss1G_013314.1 (ScyD), and Trihydroxynapthalene reductase: Ss1G_013315.1 (3 HNR)) were selected and experiments carried out to determine when these genes are 'switched on' in *S. sclerotiorum.*

PCR reactions were carried out using cDNA from *S. sclerotiorum* wild type cultivar 1980 grown on PDA + cellophane as template. Samples of *S. sclerotiorum* UQ1280 infecting *Brassica napus* (canola) were also used (data not shown). A water only and gDNA control were included to preclude contamination, genomic or otherwise, from the results.

Results and Discussion

ScyD and 3HNR were expressed in melanised mycelia and sclerotia but not in growing mycelia when *S. sclerotiorum* was grown *in vitro* (Fig 6.9). These results support the chemical inhibition data that indicate that the DHN melanin pathway is active in *S. sclerotiorum,* as described by Butler *et al.* (2009). Other work (not shown) has indicated these genes are also expressed during infection of *Brassica napus* (canola).

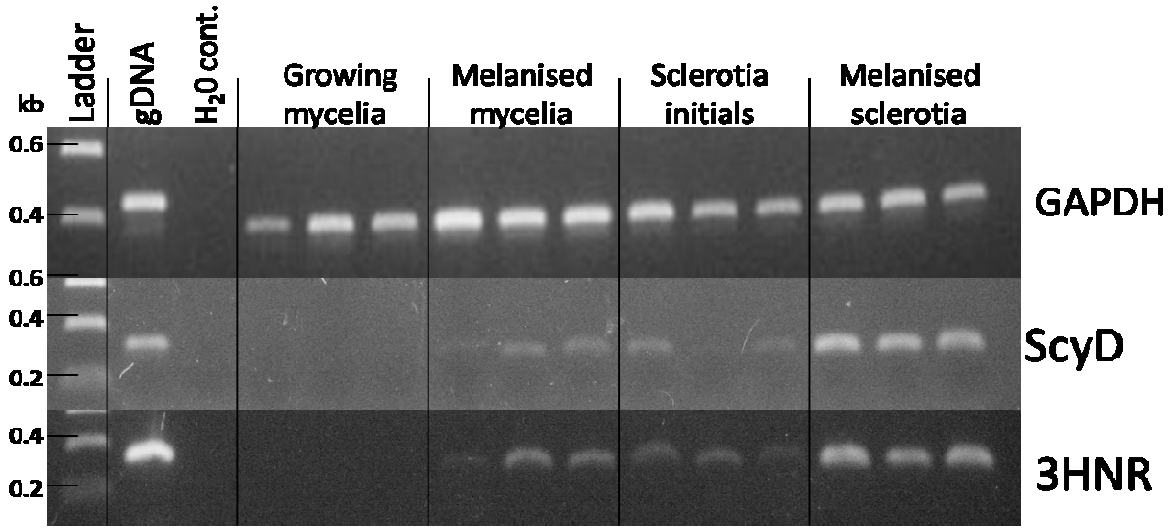


Figure 6.9. Products amplified via RT-PCR indicate expression of Scytalone dehydratase (ScyD) & tihydroxynapthalene reductase (3HNR) in mycelia & sclerotia during *S. sclerotiorum* growth *in vitro*. A gDNA control was included to demonstrate larger band size expected if products were due to gDNA contamination. Amplification specific to glyceraldehydes 3-phosphate dehydrogenase (GAPDH) transcripts indicates that the amount of cDNA in each sample is approximately even.

6.2.3 Knock out of melanin biosynthesis genes

Materials and methods

In addition to testing chemical inhibitors, the contribution of DHN melanin to proper sclerotial formation in *S. sclerotiorum* can be tested by creating a mutant that lacks the relevant genes. This is possible by use of a 'knock-out' vector, which works by replacing a region of native DNA with an antibiotic resistance cassette.

A vector targeting both ScyD (Ss1G_013314.1) and 3HNR (Ss1G_013315.1) was designed and constructed using a combination of overlap PCR and restriction digests (pFAT3-MelKO: Fig 6.10). In theory it is possible to knock out both of these genes as they are adjacent in the genome of *S. sclerotiorum.*

After the knockout vector pFAT3-MelKO (Fig 6.9) was constructed it was transformed into *Agrobacterium tumefaciens. Agrobacterium*-mediated transformation (AMT) was then attempted using a technique similar to that used in Fitzgerald *et al.,* 2004. A similar vector, missing only the ScyD and THN ends, was also transformed into *S. sclerotiorum* as an empty vector control.

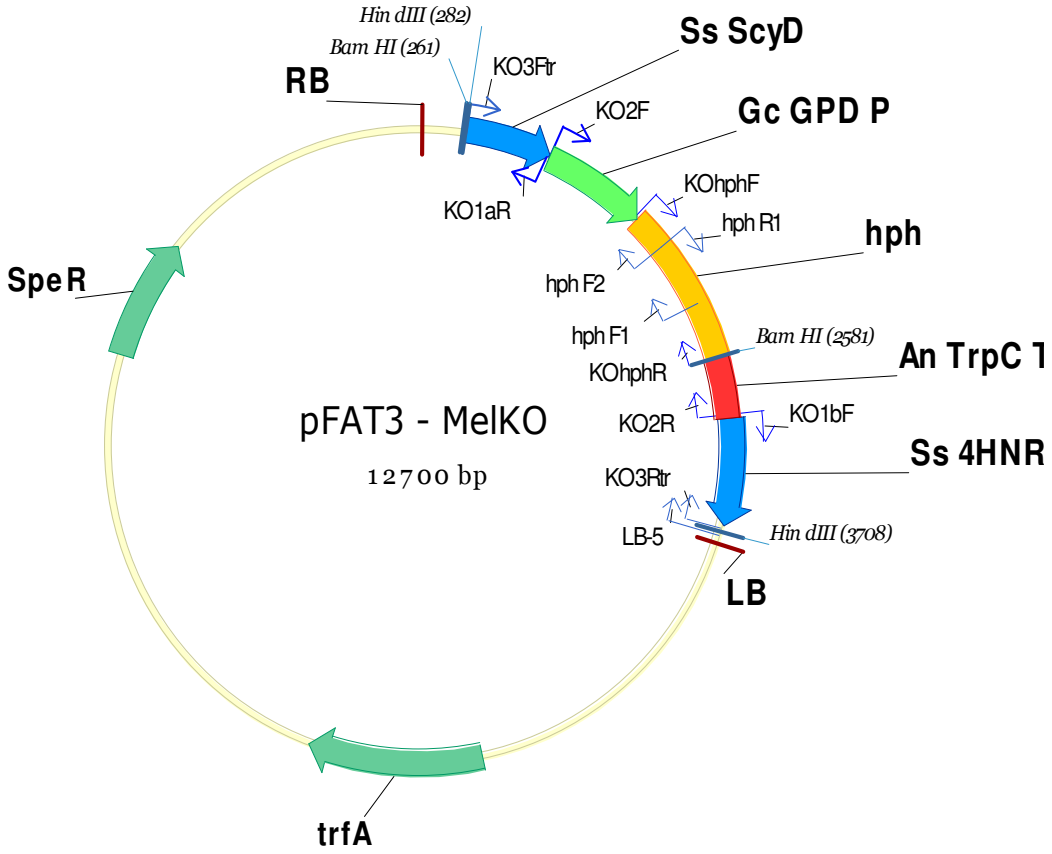


Figure 6.10 Schematic diagram showing the completed knock out vector pFAT3-MelKO

Results and discussion

The vector pFAT3-MelKO has been constructed; however successful transformation into *S. sclerotiorum* has not yet been achieved. Work is on-going to generate a reliable transformation procedure for *S. sclerotiorum* using a different vector (pFAT3-gfp) that has been used to successfully transform *S. sclerotiorum* in the past (K. Plummer, La Trobe University Botany Department, personal communication).

6.2.4 Conclusions

This fundamental research has identified the pathway by which *Sclerotinia* produces melanin an essential component of sclerotia, the survival structures of this pathogen. Chemical disruption of this pathway resulting in inhibition of sclerotial development and/or melanisation has been demonstrated. In summary this work has demonstrated:

- *Sclerotinia* produces DHN melanin in mycelia and sclerotia
- Tricyclazole, and to a lesser extent, pyroquilon, affects sclerotial formation in *Sclerotinia*
- It is possible that *S. sclerotiorum* and *S. minor* also employ another melanin biosynthesis pathway, eg: L-DOPA

6.3 Sclerotia aberrant mutants

6.3.1 Phenotyping sclerotia-aberrant mutants

Materials and methods

T-DNA mutants of *S. sclerotiorum* were created by the University of Florida via *Agrobacterium*-mediated transformation (AMT). In this process a short DNA sequence (T-DNA) is inserted randomly into the fungal genome (Fig 6.4). As the sequence of this t-DNA tag is known, the gene or region into which it has been inserted can be identified. A number of these mutants display a sclerotia-minus, or sclerotia-aberrant phenotype. These mutants were sent to La Trobe University for study as part of this project. The phenotypes of these mutants were characterised according to their phenotype on solid media, ability to produce sclerotia and organic acids, and their pathogenicity. Mutants of particular interest were further characterised by quantifying the amount of oxalic acid produced using a kit. Oxalic acid is an important pathogenicity factor that is also implicated in sclerotial formation (Godoy *et al,* 1990).

Results and discussion

Mycelial growth rate
(measured by number of days needed to fill a 9cm, 20ml PDA plate with mycelia):

- 1.1 % of mutants grow at the same rate as WT (3 days)
- 48.9 % take 1 day longer than WT (4 days)
- 25 % take 2 days longer (5 days)
- 25 % take 3 days longer (6 days)

Sclerotial production

- 2 mutants do not make sclerotia or sclerotial initials at all
- 36 % make aberrant sclerotia (incorrectly formed or melanised rind, medulla etc)
- 11 % of sclerotia producing isolates make sclerotia that do not germinate myceliogenically
- 49 % of sclerotia producing isolates take twice the amount of time WT isolates take to produce mature sclerotia in culture (WT = 15 days)

Acid production

- 1 isolate is capable of making organic acids, but not oxalic acid
- All other isolates appear to make oxalic acid

Pathogenicity

- 1 isolate has reduced pathogenicity (same mutant does not make oxalic acid)
- All other mutants appear to be pathogenic, this experiment needs to be repeated for some isolates

The phenotype experiments allowed identification of the most promising mutants (those producing aberrant sclerotia) to investigate. Field isolates of *S. sclerotiorum* that produce abnormal sclerotia have been shown to have reduced viability in soil, as parasitic organisms such as *Coniothyrium minitans* can colonise them more easily (Huang, 1982). Similarly, isolates that produce sclerotia with a brown or amber medulla also display reduced viability (Huang, 1982). Therefore these isolates are nearly as important as those that produce no sclerotia at all.

An oxalic acid-minus, non sclerotial producing, non-pathogenic mutant was identified (mutant 226). This mutant displayed a similar phenotype to another *S. sclerotiorum* mutant described in 1990 (Godoy *et al*) upon which much published work has been based. Another interesting mutant is also incapable of producing sclerotia (mutant 61), however it produces oxalic acid and is pathogenic. Study of this mutant may allow us to further elucidate the process of sclerotial formation in *Sclerotnia.*

6.3.2 Identification of T-DNA insertions points

Materials and Methods

Thermal Asymmetric Interlaced PCR (TAIL-PCR) was used to identify t-DNA insertions into mutant *S. sclerotiorum* genomes of interest (identified from phenotyping studies detailed above). In this method 3 nested T-DNA specific oligos were paired in consecutive reactions with an arbitrary degenerative (AD) oligo (non-specific) (Mullins *et al,* 2001) . The different melting temperatures of the specific and AD primers allows for thermal control of amplification efficiency of specific and non-specific products (Liu *et al*., 1995). Over the 3 consecutive reactions products containing T-DNA sequence were preferentially amplified. These products were then purified, cloned if necessary, and sent for sequencing at the Australian Genome Research Facility.

Sequences were analysed for quality using the Invitrogen program Vector NTI, then compared against data available online at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the *S. sclerotiorum* database (http://www.broadinstitute.org/annotation/genome/sclerotinia_sclerotiorum/Blast.html).

Once sequences adjacent to the t-DNA were identified work was carried out to

1. Determine significance of insertion point – ie: in a gene or promoter?
2. Validate sequencing results via PCR by designing primers that flank the insertion point
3. Establish where and when gene is expressed – eg: in mycelia, in sclerotia – either via RT-PCR or by looking up EST database

Results and Discussion

A number of T-DNA insertion points have been identified and characterised (Table 6.3). Work is continuing to determine whether these mutations are responsible for the aberrant phenotypes seen, and to identify further insertion points in other mutants. If a particular mutation can be linked to inhibition of / aberrant sclerotial formation this could provide a target for control of *Sclerotinia* via a reduction in sclerotial formation and/or survival.

Table 6.3. Identified T-DNA insertion points and other relevant information

Mutant	T-DNA inserted into	Further Information	Expressed in:
61	Gene 11086.1	Hypothetical gene, orthologs found in *Bortrytis cinerea* and *Pyrenophora tritici repentis*	Gene not expressed *in vitro* or during infection.
185	Gene 01691.1	Predicted to be an mRNApoy-adenylation factor	Gene expressed in sclerotia
196	'Intergenic'	Possibly inserted into a miscalled gene due to expression	Expressed in mycelia and sclerotia
226	Retrotransposable element	Several retrotransposable elements are present in *S. sclerotiorum.* 226 has at least one more unidentified T-DNA insertion	Data n/a
713	Promoter of 01017.1	01017.1 is a hypothetical protein with orthologs in many filamentous fungi	Data n/a
252	Gene	Gene is similar to a kelch protein (anchored to cell wall)	Expressed in mycelia
580	Promoter of 02864.1	Gene encodes an oxidoreductase	Data n/a

6.3.3 Conclusions

This study has demonstrated that mutants can be used to identify genes involved in the formation of sclerotia, the survival structures of *Sclerotinia* spp. These genes may provide targets for sustainable control of *Sclerotinia* and other soil borne plant pathogens

6.4 References

Bloomfield, B. J. a*nd Alexand*er, M. (1967) Melanins and resistance of fungi to lysis. *Journal of Bacteriology* **93, 4:** 1276 – 1280

Butler, M. J. and Day, A. W. (1998) Fungal melanins: a review. *Canadian Journal of Microbiology* **44:** 1115 – 1136

Butler, M. J., Gardiner, R. B. and Day, A. W. (2005) Degradation of melanin or inhibition of its synthesis: are these a significant approach as a biological control of phytopathogenic fungi?. *Biological Control* **32:** 326 – 336

Butler, M. J., Gardiner, R. B. and Day, A. W. (2009) Melanin synthesis by *Sclerotinia sclerotiorum. Mycologia* **101, 3:** 296 - 304

Chet, I. and Henis, Y. (1975) Sclerotial morphogenesis in fungi. *Annual Review of Phytopathology* **13:** 169 – 192

Fitzgerald, A., van Kan, J. A. L. and Plummer, K. M. (2004) Simultaneous silencing of multiple genes in the apple scab fungus, *Venturia inaequalis,* by expression of RNA with chimeric inverted repeats. *Fungal Genetics and Biology* **41:** 963 – 971

Fleet, C. and Breuil, C. (2002) Inhibitors and genetic analysis of scytalone dehydratase confirm the presence of DHN-melanin pathway in sapstain fungi. *Mycological Research* **11:** 1331 – 1339

FRAC (2009) Poster: Mode of action of fungicides: FRAC classification on mode of action. Accessed at:
http://www.frac.info/frac/publication/anhang/FRAC%20MoA%20Poster%202009_Final_LowRes.pdf
On: 22/03/2010

Godoy, G., Steadman, J. R., Dickman, M. B. and Dam, R. (1990) Use of mutants to demonstrate the role of oxalic acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris. Physiological and Molecular Plant Pathology* **37:** 179 – 191

Huang, H. C. (1982) Morphologically abnormal sclerotia of *Sclerotinia sclerotiorum. Canadian Journal of Microbiology* **28:** 87 – 91

Huang, H. C. (1983) Pathogenicity and survival of the tan-sclerotial strain of *Sclerotinia sclerotiorum. Canadian Journal of Plant Pathology* **5:** 245 – 247

Huang, H. C. (1985) Factors affecting myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum. Ecology and Epidemiology* **75, 4:** 433 – 437

Kuo, M. J. and Alexander, M. (1967) Inhibition of the lysis of fungi by melanins. *Journal of Bacteriology* **94, 3:** 624 – 629

Le Tourneau, D. (1979) Morphology, cytology and physiology of *Sclerotinia* species. *Phytopathology* **69, 8:** 887 – 890

Liu, Y. G., Mitsukawa, N., Oosumi, T. and Whittier, R. F. (1995) Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. *The Plant Journal* **8, 3:** 457 – 463

Mullins, E. D., Chen, X., Romaine, P., Raina, R., Geiser, D. M. and Kang, S. (2001) *Agrobacterium*-mediated transformation of *Fusarium oxysporum*: An efficient tool for insertional mutagenesis and gene transfer. *Phytopathology* **91, 2:** 173 - 180

Purdy, L. H. (1979) *Sclerotinia sclerotiorum:* history, diseases and symptomology, host range, geographic distribution, and impact. *Phytopathology* **69:** 875 – 880

Solomon, P. S., Tan, K.-C., Sanchez, P., Cooper, R. M. And Oliver, R. P. (2004) The disruption of a Gα subunit sheds new light on the pathogenicity of *Stagonospora nodorum* on wheat. *Molecular Plant Microbe Interactions* **17:** 456 – 466

Thompson, J. E., Fahnestock, S., Farrall, L., Liao, D.-I., Valent, B. And Jordan, D. B. (2000) The second napthol reductase of fungal melanin biosynthesis in *Magnaporthe grisea. The Journal of Biological Chemistry* **275:** 34,867 – 34, 872

Tsai, H.-F., Washburn, R. G., Chang, Y. C. and Kwon-Chung, K. J. (1997) *Aspergillus fumigatus arp1* modulates conidial pigmentation and complement deposition. *Molecular microbiology* **26:** 175 – 183

Willets, H. J. and Bullock, S. (1992) Developmental biology of sclerotia. *Mycological research* **96:** 801 – 816

Willets, H. J. and Wong, J. A. L. (1980) The biology of *Sclerotinia sclerotiorum, S. trifoliorum* & *S. minor* with emphasis on specific nomenclature. *The Botanical Review* **46:** 101 – 165

Zhou T. and Boland, G. J. (1998) Biological control strategies for *Sclerotinia* diseases.
In: Boland, G. L., Kuykendall, L. D., eds. Plant-microbe interactions and biological control. New York: Marcel Dekker Publishers. p 127–156.

Fungal derived volatiles (endophytes from native species)

7. Fungal derived volatiles (endophytes from native species)

Researchers: Ross Mann and Scott Mattner (Department of Primary Industries, Victoria)

Fungi that live in native flora (endophytes) have recently been shown to produce volatile metabolites which can be used as natural bioprotectants against pathogens (fungi, nematode and bacteria) and pests (weeds and insects) of horticultural crops. A system, called mycofumigation, has been developed that incorporates the endophyte into soil where it releases its volatile biocidal compounds to reduce pathogen and pest inoculum. Mycofumigation is showing great potential for pathogen control and offers a number of potential benefits to growers compared to synthetically derived agrichemicals. These include (i) greater sustainability (reduced likelihood of pesticide resistance), (ii) reduced environmental impacts (eg alternatives to ozone-degrading fumigants), (iii) improved worker safety (potentially less toxic), and (iv) reduced expenditure on cropping inputs (cheaper product). This project has advanced the development of this new disease management system, utilising native endophytes for Australian horticulture. It has demonstrated that:

- Australian native plants are excellent sources of endophytic fungi that produce volatile antimicrobial metabolites.
- a total of 18 endophytes (from one genus) demonstrated strong antimicrobial activity in *in vitro* bioassays, completely controlling vegetable crop pathogens such as *Pythium sulcatum* and *Sclerotinia minor* and severely restricting the growth of *Fusarium oxysporum*.
- these endophytes also demonstrated strong herbicidal activity against common horticultural weeds, severely restricting the germination of perennial ryegrass, and reducing the growth of the hard-seeded legume, vetch.
- volatile compounds produced by these endophytes included a range of common terpenes (eg eucalyptol and pinene) that are known to have antimicrobial activity, some of which are being evaluated as biocontrol strategies in horticulture (ie pine oil / pinene – chapter 5). The greatest quantity and diversity of volatile compounds was produced when the endophyte was > 10 days old.
- mycofumigation produced significantly equivalent results in pot trials to the commercially available fumigant Basamid®, reducing inoculum levels of *Rhizoctonia solani* (AG2.1) by > 99.0 %.

7.1 Introduction

Mycofumigation is the process of using volatile biocidal metabolites produced by fungi to control soilborne phytopathogens (fungi, bacteria and nematodes) and pests (weeds and insects). Recent research has identified endophytic fungi, which reside symbiotically within plant tissues, as diverse sources of volatile biocidal metabolites that they use to protect their host from pathogen and pest infection.

Endophytic fungi can produce a suite of volatile biocidal metabolites of diverse chemistries, including alcohols, acids, esters, ketones, and lipids. As such, these metabolites often have differing modes of action, and are thought to act synergistically against a wide range of organisms. For example, the endophytic fungus *Muscodor albus*, isolated from a cinnamon tree in Honduras, was found to produce

← Image previous page, chapter 7 coversheet – temperate forest, a source of endophytic fungi.

at least 28 volatile metabolites. A separate study showed that these compounds acted additively and synergistically with each other to give *M. albus* its high activity against pathogens. The US company AgraQuest have recently commercialised and registered this isolate of *M. albus* (CZ620) for use as a mycofumigant in the USA (including California) and South America.

Due to the synergistic and multi-mode nature of mycofumigation it is postulated that there is less likelihood of pests and pathogens developing resistance, compared with synthetic agrichemicals. This is because agrichemicals commonly have only one active ingredient (and one mode of action). In addition, it is widely recognised that microbes can synthesise metabolites of diverse chemical structures and activities that exceed the scope of synthetic organic chemistry. As such, compounds from microbes (including endophytic fungi) may be more likely to have new modes of action and lack cross-resistance issues compared with current synthetic agrichemicals.

Furthermore, endophytic fungi and their volatile metabolites may be less toxic than synthetic agrichemicals. The volatile metabolites of endophytes appear to specifically target lower eukaryotic and prokaryotic organisms (eg pathogens), and cause no apparent harm to their host (higher eukaryotic organisms). Therefore, it is hypothesized that endophytes and their volatile metabolites are less toxic to higher eukaryotes, including humans. Since endophytes can be produced on inexpensive, renewable substrates, they are also likely to be less costly for growers compared with some synthetic chemicals.

Australia's diverse native flora represents an untapped source of fungal endophytes, some of which may contain volatile biocidal metabolites with potential application as natural bioprotectants in horticulture. For example, new species of *Muscodor* have been isolated from Australian native plants. This chapter reports on the first Australian trials evaluating native endophytes as sources of volatile biocidal metabolites, and their application as potential mycofumigants against pathogens and weeds of vegetable crops.

7.2 Materials and method

7.2.1 Endophytes

The endophyte isolates evaluated in this chapter were collected during the DPI Victoria-funded program 'Prospecting for natural bioprotectants from endophytes of native flora' (CMI 102033). A total of 18 isolates were selected for evaluation, with all isolates contained in the same genus.

7.2.2 Genetic Diversity of Endophytes

Genomic DNA was extracted from endophyte cultures grown in potato dextrose broth (PDB) using a DNeasy Plant Mini Kit (Qiagen). A section of the ribosomal RNA locus (5.8S / ITS) was amplified with primers ITS4 and ITS5. PCR amplifications were performed in 25 µL reaction volumes containing 1.0 U of Platinum Taq DNA Polymerase (Invitrogen), x 1 PCR buffer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 0.5 µM of each primer, and 15 – 25 µg DNA. Reactions were performed in a thermocycler (Gradient Palm-Cycler, Corbett Research) with cycling conditions consisting of denaturation at 94ºC (3 min), followed by 35 cycles at 94ºC (30 s), 50ºC (30 s), and 72ºC (2 min), with a final extension step at 72ºC (3 min) to complete the reaction. PCR products were separated by electrophoresis at 100 V for 45 min in a 1.5 % (w/v) agarose gel (containing ethidium bromide, 0.1 ppm) in 0.5 X TBE running buffer and visualised under UV light. PCR products (500 – 550 base pairs) were purified using a PCR Purification Kit (Qiagen), and sequenced using the BigDye Terminator Cycle v 3.1 sequencing kit (Applied Biosystems) on the ABI 3730xl Capillary Sequencer (Applied Biosystems), according to manufactures instructions.

7.2.3 Bioactivity

Fungi

An *in vitro* bioassay was conducted to evaluate the ability of the endophytes to control three pathogens of vegetable crops (*Pythium sulcatum, Sclerotinia minor* and *Fusarium oxysporum)* through the production of volatile antimicrobial compounds (see Table 7.1). Endophytes were compared against the commercialised mycofumigant *Muscodor albus* (CZ630). Endophytes were sub-cultured onto one half of a PDA (39.0 g / L) (Amyl Media Pty Ltd) septum plate (ie agar plate with septum dividing the plate in half) by placing a 6 mm plug (with actively growing hyphae) 13 mm from the edge of the plate. Endophytes were allowed to grow for 7 days at room temperature. Subsequently, the other half of the septum plate was inoculated with a pathogen by placing a 6 mm plug (with actively growing hyphae) 13 mm from opposite edge of the plate to the endophyte. The plate was sealed with LDPE and allowed to grow at room temperature. After 5 days growth the radius of the pathogen was measured. Measurements were expressed as percentage inhibition compared with the control (no endophyte present). Data were analysed using ANOVA as performed in GenStat (version 11). The experiment was fully randomised with four replicates.

Weeds

An *in vitro* bioassay was conducted to evaluate the ability of the endophytes to control two weeds of horticultural significance (*Lolium perenne* and *Vicia sativa)* through the production of volatile antimicrobial compounds. The endophytes were compared with a fungus that produces non-biocidal volatiles, *Trichoderma* sp, as an additional control. Endophytes were sub-cultured onto one half of a PDA (39.0 g / L) (Amyl Media Pty Ltd) septum plate by placing a 6 mm plug (with actively growing hyphae) 13 mm from the edge of the plate. Endophytes were allowed to grow for 7 days at room temperature. Subsequently, ten weed seeds on a moistened cottonwool pad were placed in the other half of the septum plate. The plates were sealed with LDPE and allowed to grow at room temperature. After 7 days the germination percentage and seedling length was measured and compared with the control (no endophyte present). Data were analysed using ANOVA as performed in GenStat (version 11). The experiment was fully randomised with four replicates.

7.2.4 Analysis of Endophyte Volatile Metabolites

Volatile metabolites produced by the endophytes were analysed to identify possible sources of their antimicrobial activity. The endophytes were grown on PDA slopes (39.0 g / L) (Amyl Media Pty Ltd) in 20 ml glass vials. Vials were sealed with a screw cap lid with PTFE septum, and grown at room temperature. The volatiles were analysed at 2, 3, 4, 5, 10, 11, 12 days after inoculation.

A “Solid Phase Micro Extraction” syringe (SPME, Supelco) consisting of 50/30 divinylbenzene/carboxen on polydimethylsiloxane on a Stable Flex™ fibre was used to absorb volatiles from the head space of the vials. The SPME fibre was initially conditioned (baked) and then placed through the septum of the vial and exposed to the vapour phase. The SPME fibre was then inserted into the splitless injection port of a gas chromatograph (GC) where the contents were thermally desorbed onto a capillary column. The GC was interfaced to a mass selective detector (mass spectrometer, MS) operating at unit resolution. Initial identification of the volatiles produced by the endophyte was made through library comparison using standard chemical databases. All chemical names in this report follow the nomenclature of these databases. In all cases, uninoculated control vials were also analysed and the compounds found therein were subtracted from those appearing in the vials supporting fungal growth. Tentative identification of the fungal volatiles was based on observed mass spectral data compared with those in chemical databases, and against selected chemical standards.

7.2.5 Mycofumigation Pot Trial

A pot trial was conducted to evaluate the mycofumigation effect of the endophyte isolates. Endophytes were grown on three different substrates (wheat seed, bran:sand mixture and vermiculite). These treatments were compared with a commercial soil fumigant (Basamid®) for control of *Rhizoctonia solani* (AG 2.1) and *Pythium* spp. Substrates (sterilised twice at 121ºC for 60 mins) were inoculated

with mycelial plugs from cultures (actively growing) of the endophyte and incubated at room temperature for 21 days. The substrates growing the endophytes were added to soil (Cranbourne sand) as inoculum at a rate of 200 g / L in sealable plastic bags and mixed thoroughly. The inoculated soil was dispensed into plastic 50 mm (diameter) x 250 mm (length) tubes and placed in a random block design with five replicates of each treatment. After 14 days, the soil was placed into a sealable plastic bag and sent to SARDI (Adelaide) for quantification (using qPCR) of *R. solani* (AG2.1) and *Pythium* spp. (concentration expressed as pg DNA / g soil). Data were analysed using ANOVA as performed in GenStat (version 11).

7.3 Results and Discussion

7.3.1 Genetic Diversity of Endophytes

Genetic diversity was detected amongst the 18 isolates, and they separated into three clades based on their rRNA-ITS sequences. The most diversely related isolates were 1.2 and 1.16.

7.3.2 Bioactivity

Fungi

All endophytes showed strong levels of activity against the three pathogens of vegetable crops (*Pythium sulcatum, Fusarium oxysporum, Sclerotinia minor*) (Table 7.1). Several endophytes completely inhibited the growth of *P.sulcatum* and *S.minor*, and inhibited the growth of *F.oxysporum* by up to 46.4 %. Several endophytes also provided equivalent (or better) control of pathogens than the commercially-available (in the USA) mycofumigant, *Muscodor albus* (CZ620).

Weeds

Endophytes reduced the germination percentage and seedling growth of key horticultural weeds (*Lolium perenne* and *Vicia sativa*). All endophytes performed equivalently. Results of a representative endophyte (Isolate 1.1) are displayed in Figures 7.1 – 7.6.

Isolate 1.1 reduced the germination percentage of *L. perenne* by around 80.0 %, and restricted the growth of germinated seedlings by over 95.0 % (Figure 7.1, 7.2 and 7.3). The endophyte provided significantly greater activity against *L. perenne* than *Trichoderma* sp. and the untreated control. Isolate 1.1 did not affect the germination of *V. sativa* seeds, but inhibited the growth of seedlings by over 34.0 %, which was significantly greater than *Trichoderma* sp. and the untreated control (Figure 7.4, 7.5 and 7.6).

Table 7.1. Percent inhibition of three pathogens of vegetable crops (*Pythium sulcatum, Fusarium oxysporum* and *Sclerotinia minor*) following exposure (5 days) to volatile secondary metabolites produced by isolates of Genus 1 (blue – industry standard).

	Pythium	Fusarium	Sclerotinia
Isolate 1.1	100.0 %	46.4 %	100.0 %
Isolate 1.2	100.0 %	6.9 %	100.0 %
Isolate 1.3	100.0 %	29.4 %	100.0 %
Isolate 1.4	89.0 %	21.6 %	95.0 %
Isolate 1.5	100.0 %	20.6 %	100.0 %
Isolate 1.6	100.0 %	10.2 %	95.2 %
Isolate 1.7	100.0 %	36.1 %	100.0 %
Isolate 1.8	100.0 %	28.9 %	100.0 %
Isolate 1.9	54.5 %	2.9 %	44.7 %
Isolate 1.10	100.0 %	27.2 %	100.0 %
Isolate 1.11	100.0 %	29.2 %	100.0 %
Isolate 1.12	100.0 %	25.9 %	100.0 %
Isolate 1.13	100.0 %	29.0 %	100.0 %
Isolate 1.14	100.0 %	0.4 %	69.5 %
Isolate 1.15	100.0 %	29.4 %	98.4 %
Isolate 1.16	54.2 %	0.0 %	62.5 %
Isolate 1.17	68.8 %	5.9 %	64.4 %
Isolate 1.18	100.0 %	9.8 %	96.3 %
Muscodor albus (CZ620)	100.0 %	32.3 %	100.0 %
LSD (5 %)	12.6	7.4	13.5
P Value	0.001	0.001	0.001

7.3.3 Analysis of Endophyte Volatile Metabolites

All endophytes were found to produce an array of volatile metabolites over a 12 day period. Many of these metabolites were common to all endophytes. Results of a representative endophyte (Isolate 1.1) are displayed in Figure 7.7.

Approximately 30 volatile compounds were produced by Isolate 1.1 over the 12 day growth period, including a number of common terpenes (eg eucalyptol and pinene), a range of cyclohexane derivatives (eg 2-Cyclohexen-1-one, 2-(2-methyl-2-propenyl)-), and ethanol (Figure 7.7). The compounds produced in highest quantities varied depending on the age of the culture. Eucalyptol was produced in highest quantities when the culture was between 2 – 10 days, with the cyclohexane derivatives only found in low quantities. Conversely, the cyclohexane derivatives were highest when the culture was > 10 days old, while quantities of eucalyptol were low at this point. The greatest diversity of compounds was produced when the culture was > 10 days old.

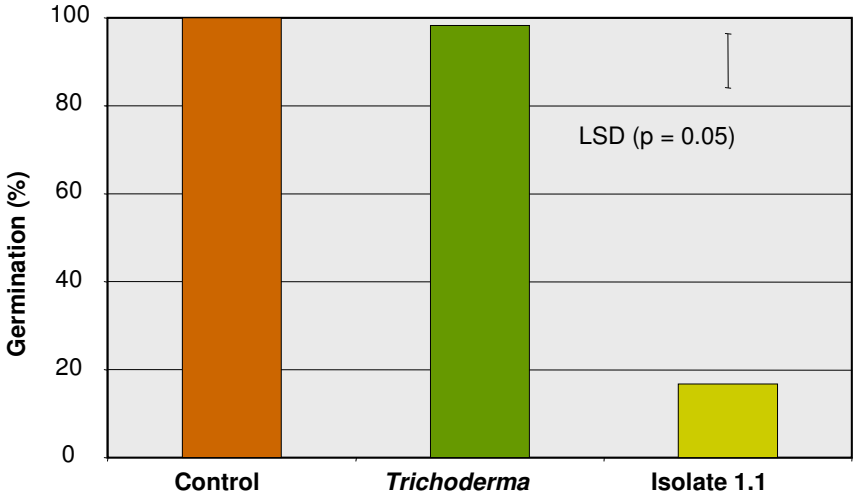


Figure 7.1. The effect of Isolate 1.1 on the germination percentage of *Lolium perenne*, compared with *Trichoderma* sp. and an untreated control.

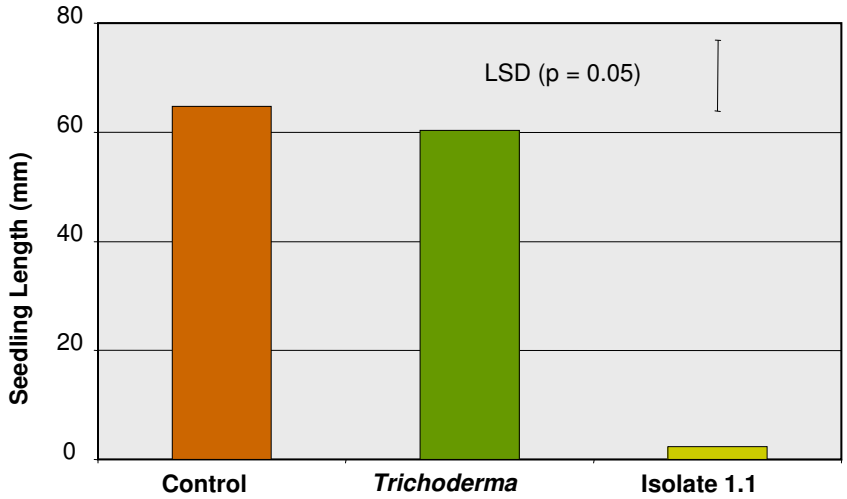


Figure 7.2. The effect of Isolate 1.1 on the seedling length of *Lolium perenne*, compared with *Trichoderma* sp. and an untreated control.

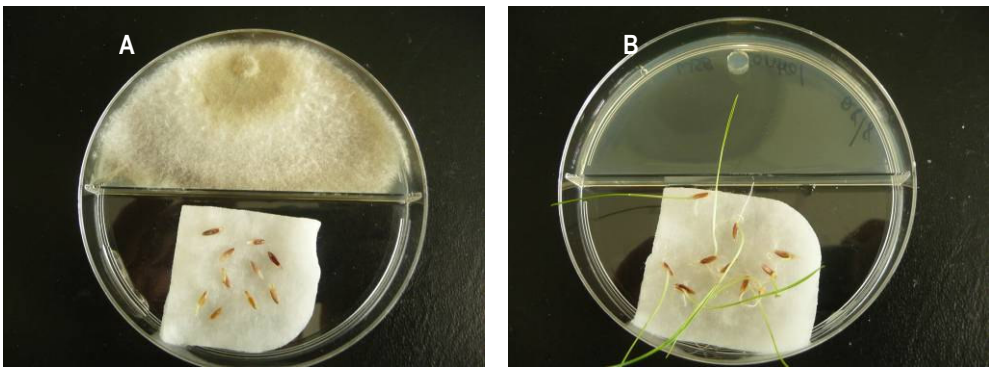


Figure 7.3. Images of *in vitro* biossays demonstrating the herbicidal activity of Isolate 1.1 (A) volatile metabolites against *Lolium perenne*, compared with an untreated control (B)

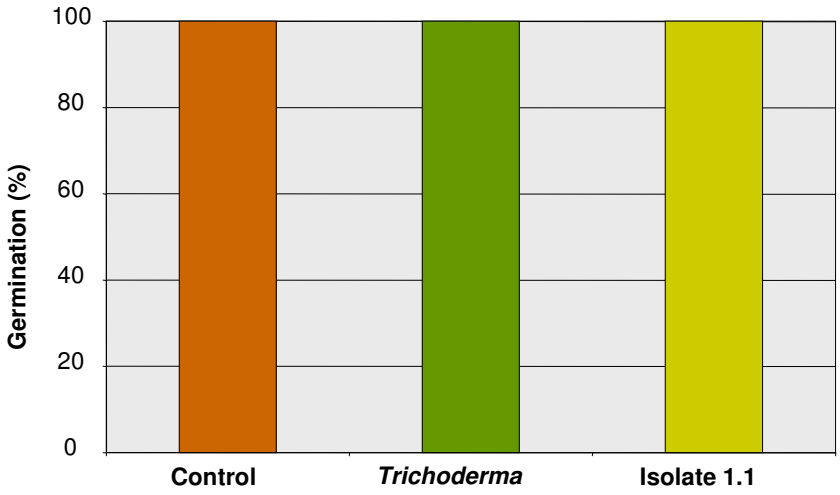


Figure 7.4. The effect of Isolate 1.1 on the germination percentage of *Vicia sativa*, compared with *Trichoderma* sp. and an untreated control.

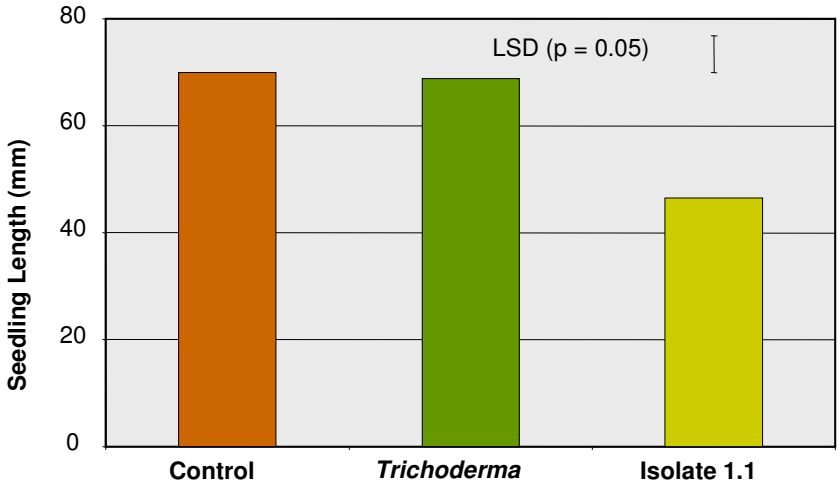


Figure 7.5. The effect of Isolate 1.1 on the seedling length of *Vicia sativa*, compared with *Trichoderma* sp. and an untreated control.

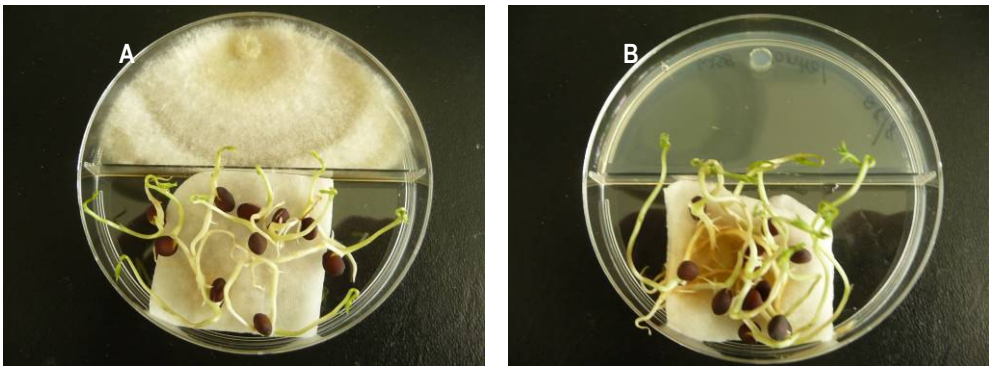


Figure 7.6. Images of *in vitro* biossays demonstrating the herbicidal activity of Isolate 1.1 (A) volatile metabolites against *Vicia sativa*, compared with an untreated control (B)

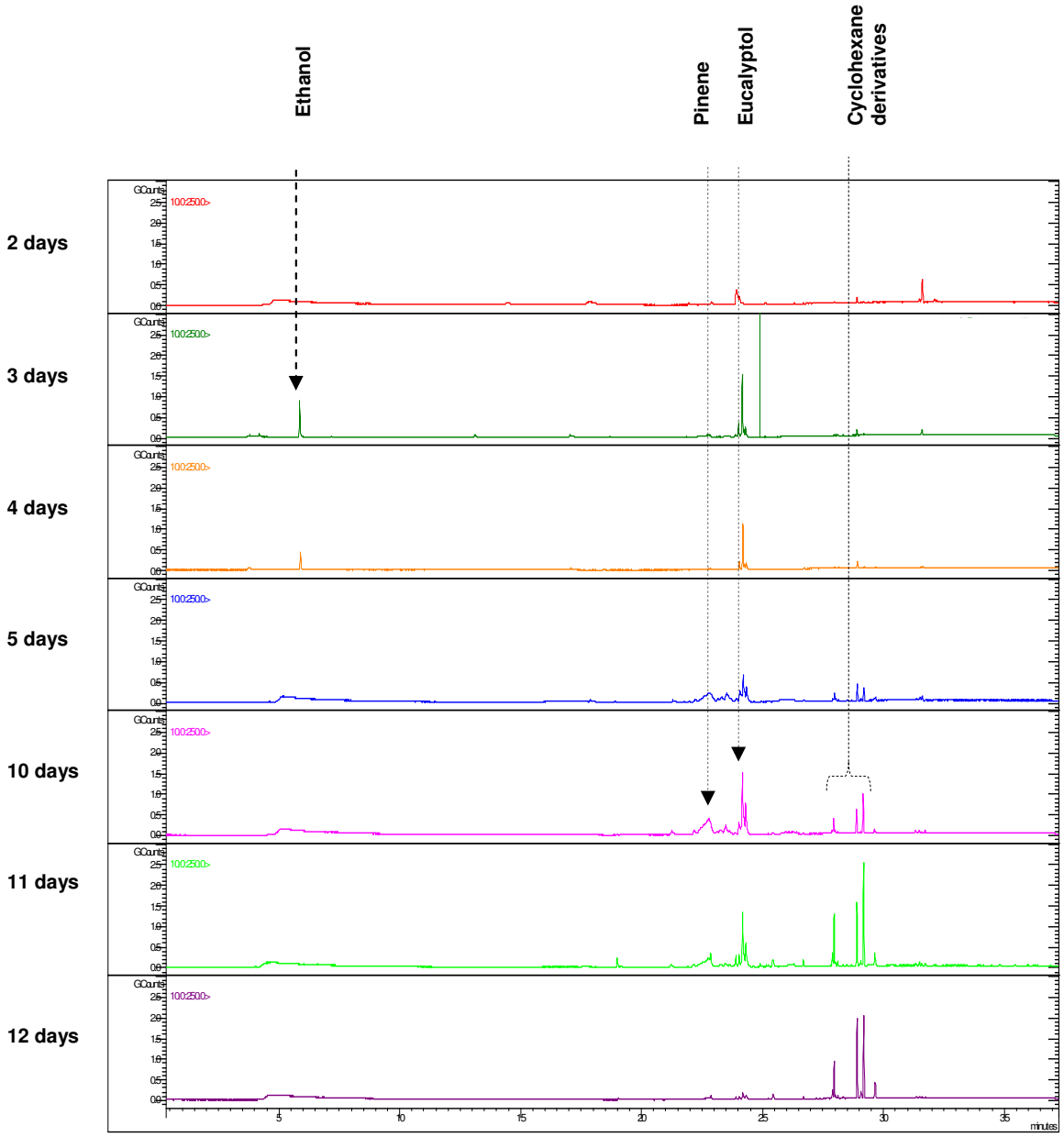


Figure 7.7. GC/MS profile of volatile compounds produced by Isolate 1.1 over a 12 day period (peaks relate to individual compounds; y axis relates to concentration of those compounds).

7.3.4 Mycofumigation Pot Trial

Pot trials showed that mycofumigation with endophytes controlled *Pythium* spp. and *Rhizoctonia solani* in soil to the same level as the synthetic fumigant Basamid®. All endophyte isolates performed equivalently. Results of a representative endophyte (Isolate 1.1) are shown in Table 7.2.

Mycofumigation with Isolate 1.1 reduced inoculum of *Pythium* spp. and *Rhizoctonia solani* (AG2.1) in soil by up to 99.9 % (Table 7.2). The optimal substrate for Isolate 1.1 was wheat seed, which provided significantly greater control of the pathogens than bran : sand (1:1) and vermiculite. Mycofumigation with Isolate 1.1 (wheat seed substrate) controlled *Pythium* spp. and *R.solani* (AG2.1) to equivalent levels as the synthetic fumigant Basamid®, reducing the concentration of *Pythium* spp. by 63.7 % and *R.solani* (AG 2.1) by 99.9 %.

Table 7.2. Effect of mycofumigation with Isolate 1.1 on populations[#] of *Pythium* spp. and *Rhizoctonia solani* (AG 2.1) in soil (blue – synthetic fumigant)

[#] - pathogen populations in soil represented by concentrations of DNA

Substrate	*Pythium* spp. (pg DNA / g soil)	*Rhizoctonia solani* (AG 2.1) (pg DNA / g soil)
Untreated	179	10,581
Bran : Sand (1:1)	271	11,986
Vermiculite	285	15,846
Wheat	65	4
Basamid®	4	0
P Value	0.004	<0.001
LSD (5 %)	131.5	5571.3

7.4 Conclusions

Laboratory and pot trials demonstrated the mycofumigation potential of native Australian endophytes against a range of significant soil-borne pathogens (*Pythium sulcatum, Sclerotinia minor, Fusarium oxysporum* and *Rhizoctonia solani*) and weeds (*Lolium perenne* and *Vicia sativa*) of vegetable crops. These endophytes were found to produce a wide variety of volatile metabolites (~ 30 compounds), some of which are known to have biocidal activity (eg eucalyptol and pinene) against pests. The biocidal activity of these endophytes is most likely due to the synergistic effect of their diverse metabolites, as is the case for the commercially available mycofumigant *Muscodor albus*. This should minimise the development of resistance in pest populations. Phenotypic (bioactivity) and genetic diversity was detected amongst isolates, but there was no direct correlation determined between these factors. Trials also demonstrated that soil mycofumigation with endophytes could reduce inoculum in soil to equivalent levels as the synthetic fumigant Basamid®.

While these trials demonstrate the potential of mycofumigation, further testing is required to (i) optimise the delivery mechanism (endophyte substrate, rates, soil sealing technologies, plant back times), (ii) determine the optimum field conditions for mycofumigation (eg soil type, soil moisture, soil chemistry, temperature), and (iii) ensure there are no environmental (eg risk of endophytes causing crop disease, note preliminary trials have demonstrated this is not the case) and human risks (eg toxicology of unknown metabolites) associated with using endophytes.

Preliminary evaluation of other novel compounds

8. Preliminary evaluation of other novel compounds

Researchers: Cherie Gambley, Lynette Haselgrove, Dalphy Harteveld, Emma Ballard, Joy Conroy (Queensland Department of Employment, Economic Development and Innovation)

Pythium spp. cause lethal seedling diseases and are a key component of root rot complexes in many vegetable crops. The work reported in this chapter evaluates a range of novel strategies with potential to manage *Pythium* spp. These strategies including use of plant derived volatiles, nutrients (silicon and potassium), surfactants and systemic acquired resistance (SAR) inducers were identified through reviews undertaken at the beginning of the project (see chapter 1). Their effects are reported here specifically relating to the control of seedling diseases in soil-based growing systems caused by *Pythium* spp. The effects of some of these strategies are evaluated elsewhere against other pathogens (see chapter 5) or in other growing systems (for example hydroponics, see chapter 3). The work has demonstrated:

- that a number of plant derived essential oils and their active components inhibit growth of *P. aphanidermatum* and *P. ultimum in-vitro*. Among the most effective were clove bud, thyme and wintergreen oils. A similar result was reported for clove bud and thyme against other soilborne pathogens in chapter 5.
- a significant reduction in the percent of beetroot seedlings affected by *P. ultimum* following treatment of sterile field soil with ECO-V®, Vigor®, Clove and Fennel in one pot trial. This result supports findings from Victorian pot (clove oil) and field (ECO-V® and Vigor®) trials (chapter 5), however, as overall disease development in Queensland trials was low ideally this work should be repeated.
- an inhibitory effect of high rates of silicon and potassium based products on in-vitro growth of *P. aphanidermatum* and *P. ultimum*. However, this did not translate to improved disease control in subsequent pot trials.
- that none of the three surfactant products evaluated significantly inhibited in vitro growth of *P. aphanidermatum* or *P. ultimum*, Agral® and DuWett® did significantly decrease disease rates in one pot trial using sterilised field soil.

8.1 Introduction

In Australia several vegetable crops are affected by *Pythium* spp. which typically cause lethal seedling diseases. The efficacies of various soil amendment products for the control of these seedling diseases were evaluated in a series of *in vitro* assays and pot trials. The products could be grouped broadly as plant derived compounds (with biofumigant activity), systemic acquired resistance (SAR) enhancers, nutrients and surfactants.

Mineral nutrition is an important factor in the production of healthy plants and the review by Datnoff *et al* (2007) shows that nutrients can have either a positive or negative effect on the development of diseases in plants. One nutrient in particular, silicon, has been identified as having a role in enhancing defence responses in plants but it may also have a direct effect on plant pathogens. Previous studies have shown that potassium silicate can inhibit the *in vitro* growth of mycelia for a number of different pathogens, including *Pythium* spp. (Bekker *et al.* 2009; Bekker *et al.* 2006). Cherif and Belanger (1994) showed that 100 and 200 ppm soluble silicate was useful in hydroponic systems to decrease disease incidence, but although disease was reduced, plants were still observed to be infected with *Pythium*. Further work identified that 100 ppm of soluble silicon increased the defence responses of plants grown

← Image previous page, chapter 8 coversheet – pot trials conducted in Queensland.

in hydroponic systems (Cherif *et al.* 1994). However, the product used was Kasil® which also contains potassium and the role of potassium, if any, in the increased defence responses was not investigated.

Potassium is reported to decrease disease caused by a range of fungal pathogens including Pythium but increase it with others, such as *Rhizoctonia solani* (Datnoff *et al.* 2007). Products such as Kasil® mentioned above contain both potassium and silicon and it is possible that the use of these products could decrease disease caused by one pathogen whilst increasing that caused by another. The role that a combination of potassium and silicon has in disease suppression is poorly understood.
Enhancing defence responses to plant pathogens using synthetic elicitors to initiate systemic acquired resistance (SAR), was shown to be effective in controlling plant diseases in several studies (Vallad and Goodman 2004). The commercially available Bion® (Syngenta) is used in Australia by the cotton industry as a seed coat. Drenching cotton seed with Bion® 500 WG was found to be effective at controlling *Thielaviopsis basicola* (Mondal *et al.* 2005).

Many soilborne plant pathogens, including *Pythium* spp., *Olpidium* spp. and *Phytophthora* spp., produce zoospores which are highly mobile in free water and capable of causing rapid disease spread. Studies have shown that surfactants are effective against zoospores produced by these plant pathogens (Stanghellini and Tomlinson, 1987; Tomlinson and Faithfull, 1980). When 20 µg/ml of Agral® was used in a hydroponics system, *Pythium* zoospore and vesicle formation were inhibited and zoospores were observed to have decreased motility (Stanghellini and Tomlinson, 1987). However, not all plant pathogenic *Pythium* species rely on zoospores as their major dissemination mechanism, thus the efficacy of such products on these pathogens is not well understood.

In this study, the efficacies of various soil amendment products for the control of seedling diseases induced by *Pythium* sp. were evaluated using *in vitro* assays and pot trials. The study concentrated on the control of *Pythium ultimum* and *P. aphanidermatum*. These two pythium species fall into very different groups within the *Pythium* genus. *P. aphanidermatum* produces zoospores typically at temperatures of 25-30°C, is considered to have a very wide host range and an optimal temperature for disease development of 30-35°C (Van der Plaats-Niterink 1981). By contrast, most isolates of *P. ultimum* don't produce zoospores and infect at cooler temperatures of around and below 20°C (Van der Plaats-Niterink 1981).

8.2 Materials and Methods

8.2.1 Products evaluated

The products evaluated for efficacy against *Pythium* are listed in Table 8.1.

Table 8.1. Name, proposed mode of action and supplier of products evaluated for efficacy against diseases caused by *Pythium* spp. (PDC refers to Plant Derived Compound).

Product	Mode of action	Supplier
Basamid®	Fumigant	Certis Australia Pty Ltd
ECO-V®	PDC - biofumigant	Prem Akhil
Fumarfert®	PDC - biofumigant	Organic Crop Protectants
Vigor®	PDC - biofumigant	Champon USA
Eugenol	PDC - biofumigant	Sigma Pty Ltd
Geraniol	PDC - biofumigant	Sigma Pty Ltd
Carvacrol	PDC - biofumigant	Sigma Pty Ltd
Black Pepper Oil	PDC - biofumigant	Sydney Essential Oils
Thyme linalool oil	PDC - biofumigant	Sydney Essential Oils
Clove bud oil	PDC - biofumigant	Sydney Essential Oils
Fennel sweet oil	PDC - biofumigant	Sydney Essential Oils
Cumin oil	PDC - biofumigant	Sydney Essential Oils
Wintergreen oil	PDC - biofumigant	Sydney Essential Oils
Kasil 2040®	SAR/ nutrient	PQ Australia Pty Ltd
Bion 50 WG®	SAR	Syngenta
Stand SKH™	SAR/nutrient	Agrichem Pty Ltd
K-carb-35™	nutrient	Nutri-tech Solutions
Silvine®	SAR/nutrient	Peter English, NSW
Du-Wett®	surfactant	Landmark
Hortiwett®	surfactant	Yara Nipro Pty Ltd
Agral®	surfactant	Farmcraft

Kasil® and Stand SKH™ are mixtures of both silicon and potassium in approximately similar proportions. Kasil® contains in addition to other minor components, *ca* 27 % SiO_2 and 13 % K_2O and Stand SKH™ *ca* 20 % SiO_2 and 15 % K_2O. To evaluate both silicon and potassium separately, Silvine® (acidulated olivine) which contains no potassium and K-carb-35™ which contains no silicon were also included in the trials.

8.2.2 In vitro assays

To select plant derived products for evaluation in pot trials, products were evaluated at two concentrations using *in vitro* assays. The products were tested against both *P. ultimum* and *P. aphanidermatum*. Each product was evaluated by inoculating Potato Carrot Agar (PCA, 20 g/L potato and 20 g/L carrot) plates containing 250 or 500 ppm of plant derived product. The plates were prepared by diluting the products either 1:20 or 1:10 (250 and 500 ppm respectively) in dimethlysulphoxide (DMSO) and adding 100 µL of the dilution to 20 mls of molten media. Two replicate plates were prepared for each treatment combination. Plates were inoculated with one 5 mm diameter section of a 7-14 day old culture then incubated at room temperature. The plates were monitored for mycelia growth and final observations were made after one week. Control plates were prepared which either contained no additive or contained 100 µL of DMSO per 20 mL of media.

To evaluate surfactant, nutrient and SAR products for inhibition of *P. ultimum* and *P. aphanidermatum* mycelia growth, the products were filter sterilised then incorporated into PCA media at a range of different concentrations. Each product was evaluated using three duplicate plates. Each plated was inoculated with two 5 mm diameter sections of 7-14 day old culture incubated at room temperature and mycelia growth measured after 24 h. Silvine® was not evaluated in these assays as it is not soluble in water.

8.2.3 Pot trial general methods

For all pot trials, three replicate pots were prepared for each treatment and five to six seeds planted per pot. Thiram treated seeds of beetroot and bean were used. The maximum and minimum temperatures for the duration of the trial were obtained from either the Applethorpe Bureau of Meteorology station for the pot trials using granite-based soil or by using a data recorder located within the glasshouse for all other trials data. Pots were lightly watered daily to maintain moist-wet soil and monitored weekly for seed germination and disease development. All trials except one compared products using field soil before and after autoclaving. The exception was the second pot trial established to evaluate the efficacy of potassium and silicon products on the control of disease in bean caused by *P. aphanidermatum*. In this trial pasteurised potting mix was used instead of field soil.

Isolations of Pythium from affected seeds/seedlings was done from representative samples from all pot trials using PCA media containing with either streptomycin at 0.1 mg/ml or 0.1 mg/ml pentachloronitrobenzene (PCNB), 0.02 mg/ml ampicillin and 15 µg/ml pimaricin.

8.2.4 Pot trials for biofumigant evaluation

Two separate pot trials were conducted to evaluate the efficacy of various plant derived products on the control of disease in beetroot caused by *P. ultimum*. The first trial was established using a granite-based field soil collected from the Stanthorpe vegetable growing region whereas the second trial was similarly prepared but used a heavy clay-based black earth soil sourced from the Lockyer Valley growing region. The rates of products used in these trials are listed in Table 8.5.

Inoculum was prepared as by blending 58 plates of 14-21 day old PCA cultures in distilled water to a final volume of 2250 mL using a waring blender. The inoculum was added to the soil at rate of 10-15 mL per pot and mixed, then the total inoculated soil for each treatment placed in a plastic bag. The test products were then added to the inoculated soil and the plastic bag sealed for 7 days after which the soil was placed into pots and left for two weeks to allow volatiles to dissipate prior to planting seeds.

8.2.5 Pot trials for evaluation of nutrient, SAR and surfactant products

The efficacy of Bion® and potassium, silicon and surfactant products to control disease of beetroot caused by *P. ultimum* was evaluated in three separate pot trials. The first trial used the granite-based soil described above with the same method of inoculum preparation and inoculation. In this trial the products were added to the plastic bags containing the inoculated soil and left for 48 h prior to distributing it into pots. The second trial was similarly prepared but used the heavy clay-based soil sourced from the Lockyer Valley growing region.

The third trial also used the clay-based soil but differed in inoculation method and product application. The inoculation method used in this trial represents a more stringent evaluation of the products through the use of more inoculum. The amount of inoculum used was approximately two-fold more in total per pot and was localised around the seedling emergence zone instead of an even distribution through the entire pot that was used previously. In this trial inoculum was prepared by blending 95 plates of 14-21 day old Pythium cultures in distilled water to a final volume of 3300 mL using a waring blender and 25 ml of inoculum added to each pot. The inoculum was mixed into the surface soil of each pot to a depth of 3-5 cm. Each treatment was then applied at a volume of 25 ml per pot and the pots lightly watered. The products were then reapplied three days later then again a further two days on.

The products were also evaluated for efficacy against disease of bean caused by *P. aphanidermatum*. Duplicate pot trials were established as described for the first and third trials used to evaluate disease control in beetroot with the exception that for the second bean trial pasteurised potting mix was used instead of the clay-based soil used in the third beetroot trial.

8.2.6 Seed treatments with nutrient and SAR products

The efficacy of nutrient and SAR products as seed treatments to control disease of beetroots caused by *P. ultimum* and disease of bean caused by *P. aphanidermatum* was evaluated using the granite-based soil described above. The inoculum and inoculation method is as described for the first beetroot pot trial used to evaluate the biofumigant products. In this trial, seeds were soaked in the test products for 5 h at room temperature in the dark, drained and air-dried for 10-30 min then either planted immediately or stored for 12 h in a sealed container prior to planting.

8.2.7 Statistical analyses

Results of all trials were evaluated using a one-way Analysis of Variance (ANOVA) with randomised blocks where treatment replicates were used as the blocks and multiple comparisons were completed using the Fisher's unprotected equation for a least significant difference of 0.05.

8.3 Results

8.3.1 In vitro assays

Several of the plant derived (biofumigant) products were effective in limiting the *in vitro* growth of both *P. aphanidermatum* and *P. ultimum*. All products, with the exception of black pepper oil and fennel sweet oil, severely inhibited the growth of both oomycetes when incorporated into the culture media (Table 8.2).

Table 8.2 Growth of *P. ultimum* and *P. aphanidermatum* shown in millimetres (mm) on *in vitro* culture media incorporating various biofumigant products.

Treatment	***P. aphanidermatum* growth (mm)**		***P. ultimum* growth (mm)**	
	250 ppm	500 ppm	250 ppm	500 ppm
DMSO[1]	40.5 d[2]	40.5 b	43.0 c	43.0 c
Control	42.0 d	42.0 b	45. 0 c	45.0 c
Eugenol	0.0 a	0.0 a	0.0 a	0.0 a
Geraniol	11.0 ab	0.0 a	0.0 a	0.0 a
Carvacrol	0.0 a	0.0 a	0.0 a	0.0 a
Black pepper oil	42.0 d	24.5 b	28.5 b	30.0 b
Clove bud oil	11.0 ab	0.0 a	0.0 a	0.0 a
Fennel sweet oil	38.0 cd	32.5 b	34.0 bc	0.0 a
Cumin oil	22.0 bc	0.0 a	2.5 a	0.0 a
Thyme linalool oil	0.0 a	0.0 a	0.0 a	0.0 a
Wintergreen oil	15.0 ab	0.0 a	0.0 a	0.0 a

[1]DMSO = dimethlysulphoxide used as a diluent for biofumigant products.
[2]Values were used for analysis of variance calculations and letters in common represent no significant difference ($P<0.05$).

Several of the nutrient products were effective at limiting the *in vitro* growth of both *P. aphanidermatum* and *P. ultimum* but only when incorporated into the media at the very high concentrations of 10 000 or 20 000 ppm (Table 8.3). In the first experiment Kasil®, Stand SKH™ and K-carb-35™ were evaluated at concentrations of 50, 500 and 1000 ppm. No significant differences were observed between growth at these concentrations and the control plates (results not shown). However in the second trial, Kasil® and Stand SKH™ were shown to be somewhat inhibitory at 1000 ppm (Table 8.3). The influence on the pH of culture media from incorporation of these nutrients was not tested; however, results published by Bekker *et al* (2006) indicate pH is not an important factor inhibiting the growth of *Pythium.*

Table 8.3. Growth of *P. ultimum* and *P. aphanidermatum* shown in millimetres (mm) on *in vitro* culture media incorporating various silicon and potassium products.

Treatment	*P. aphanidermatum* growth (mm)	*P. ultimum* growth (mm)
Control (untreated)	41.7 c	41.2 b
Kasil® 2040 at 1000 ppm Si	26.2 b	11.0 c
Kasil® 2040 at 10 000 ppm Si	0.0 a	0.0 a
Kasil® 2040 at 20 000 ppm Si	3.3 a	0.0 a
Stand SKH™ at 1000 ppm Si	29.0 b	9.2 c
Stand SKH™ at 10 000 ppm Si	0.0 a	0.0 a
Stand SKH™ at 20 000 ppm Si	0.0 a	0.0 a
K-carb-35™ at 1000 ppm K	41.2 c	41.8 b
K-carb- 35™ at 10 000 ppm K	0.0 a	0.0 a
K-carb-35™ at 20 000 ppm K	0.0 a	0.0 a

The three surfactant products, Du-Wett®, Hortiwett® and Agral® were evaluated for inhibition of oomycete growth at 20 and 50 µg/ml using *in vitro* assays. None of the three products were effective in significantly reducing the *in vitro* growth of *P. aphanidermatum* or *P. ultimum* at either concentration (results not shown).

8.3.2 Pot trials for evaluation of plant derived products

The efficacy of several plant derived (biofumigant) products on the control of disease in beetroot by *P. ultimum* was evaluated using two different field soils in two separate trials. Both trials used similar inoculation and product application methods. The average minimum and maximum temperatures for the trials were very similar, around 14°C and 27°C, respectively. The results from these two trials are summarised in Table 8.4.

In the first trial, germination was variable in the non-sterilised soil but this was not necessarily attributable to the presence of *Pythium* as there was also a low germination rate in the non-inoculated control treatment. However, germination rates were significantly enhanced by the use of Basamid®, ECO-V® and fennel oil (Table 8.4). Although more uniform, germination rates in the sterilised soil were again significantly improved by the use of Basamid® and fennel oil. There were no significant differences observed between the efficacies of the treatments to control disease amongst seedlings using non-sterilised soil. This is mainly attributable to a lack of disease development in the control plants. By contrast, more disease was observed in seedlings grown in this soil after it was sterilised but no treatments resulted in significantly improved disease control. Instead, use of clove oil increased disease incidence.

The pot trial was repeated using the heavy clay-based soil instead of the granite based soil used in the first experiment. In this trial high germination rates were observed for all treatments, irrespective of soil sterilisation (results not shown). Again there was a low level of disease development in the inoculated control plants. Most treatments gave similar disease levels as the control plants with the exception of Fumafert® which significantly increased disease development and clove oil which gave slightly elevated disease levels (Table 8.4). By comparison, when this soil was used after sterilisation a high level of disease developed in the inoculated control plants and most products gave significantly improved disease control as compared to this control. The exceptions to this were the use of Fumafert® and thyme oil which resulted in similar disease levels as the inoculated control. Interestingly, the use of clove oil in the sterilised soil did not enhance disease as was observed when used to treat the non-sterilised soil in this trial and the sterilised soil in the previous trial.

Unexpectedly, the use of fennel oil generally resulted in less disease than when clove oil was used as the soil biofumigant. This is in contrast with results from *in vitro* assays where mycelia growth was inhibited more by clove than it was by fennel oil (Table 8.2).

Table 8.4. The efficacy of various biofumigant products in the control of *P. ultimum* induced disease of beetroot. Efficacy was evaluating the percent of seed germination (G) and/or the percent of seedlings affected (A). This was done for non-sterilised (NS) and sterilised (S) soil.

Treatment	Trial 1				Trial 2	
	G NS (%)	G S (%)	A NS (%)	A S (%)	A NS (%)	A S (%)
Uninoculated control	53.3 a[1]	63.3 a	0.0 a	2.8 ab	0.0 a	0.0 a
Inoculated control	60.0 a	66.7 a	4.2 a	9.5 abc	3.7 a	33.0 c
Basamid® (50 g/m^2)	100.0 c	100.0 b	3.3 a	5.2 ab	0.0 a	3.3 ab
ECO-V® (1 % v/v)	86.7 bc	70.0 a	0.0 a	11.1 abc	7.0 a	4.2 ab
Fumafert ® (500 kg/ha)	66.7 ab	66.7 a	8.3 a	12.0 abc	39.1 b	20.7 bc
Vigor® (10 L/ha)	60.0 a	60.0 a	4.8 a	9.6 abc	8.3 a	4.2 ab
Thyme (500 ppm)	66.7 ab	73.3 a	0.0 a	0.0 a	NT	NT
Thyme (1000 ppm)	NT[2]	NT	NT	NT	7.9 a	13.9 abc
Clove (500 ppm)	53.3 a	70.0 a	12.5 a	21.5 c	NT	NT
Clove (1000 ppm)	NT	NT	NT	NT	18.0 ab	3.3 ab
Fennel (500 ppm)	100.0 c	96.7 b	0.0 a	15.8 bc	NT	NT
Fennel (1000 ppm)	NT	NT	NT	NT	3.7 a	4.2 ab

[1]Values were used for analysis of variance calculations and letters in common represent no significant difference ($P<0.05$).
[2]NT= not tested

8.3.3 Pot trials to evaluate nutrient and SAR products

The efficacy of nutrient and SAR products on the control of disease in beetroot by *P. ultimum* was evaluated using two different field soils in separate trials. The first two trials used similar inoculation and product application methods. The results are summarised in Table 8.5. The average minimum and maximum temperatures for the two trials were very similar, around 14°C and 27°C, respectively. The third trial differed from these as it used more inoculum, the products were applied three times instead of just once at planting and the average minimum and maximum temperatures were higher, 21°C and 34°C, respectively. The results for the third trial are summarised in Table 8.6.

For the first and second pot trials, germination was relatively uniform in both the sterilised and non-sterilised soil with most treatments giving good germination rates (results not shown). In the first trial, using granite-based soil, there were no significant differences between the efficacies of the products to control *Pythium* disease amongst seedlings using non-sterilised soil (Table 8.5). This is mainly attributable to a lack of disease development in the control plants. By contrast, more disease was observed in seedlings grown in this soil after it was sterilised but none of the products resulted in significantly improved disease control, instead all were observed to have the reverse affect (Table 8.5).

In the second trial a clay-based soil was used and different rates of the products were tested, although the application method remained the same as the first trial. No product gave a statistically significant

improvement to disease control as compared to the inoculated control when used to treat non-sterilised soil but Silvine® (500 ppm Si), K-carb-35™ (50 and 400 ppm), Kasil® (5000 ppm) and Bion® (2.5 µg/ml) did reduce disease levels below that observed for the inoculated control. Of the remaining products, Silvine® (1000 ppm), K-carb-35™ (200 ppm) and stand Stand SKH™ (5000 ppm) resulted in elevated disease levels. Relatively uniform levels of disease were observed for all products when used to treat sterilised soil. The exceptions to this were K-carb-35™ at 200 ppm which resulted in elevated disease levels and Silvine™ used at 500 and 1000 ppm which gave slightly reduced disease levels as compared to the inoculated control.

Table 8.5. The efficacy of nutrient and SAR products in the control of *P. ultimum* induced disease of beetroot as determined in pot trials 1 and 2. Efficacy was evaluated as the percent of seedlings affected (A). This was done for non-sterilised (NS) and sterilised (S) soil.

	Trial 1		Trial 2	
Treatment	A NS (%)	A S (%)	A NS (%)	A S (%)
Uninoculated control	3.0 a[1]	2.1 a	3.7 a	0.0 a
Inoculated control	3.2 a	8.8 ab	18.5 ab	36.4 ab
Kasil® 5000 ppm Si	8.3 a	23.0 b	10.4 a	30.4 ab
Bion® 25 µg/ml	6.7 a	18.1 b	17.3 ab	24.3 ab
Bion® 2.5 µg/ml	NT[2]	NT	11.7 a	37.7 ab
Bion® 0.25 µg/ml	NT	NT	18.5 ab	32.1 ab
Stand SKH™ 5000 ppm Si	5.6 a	18.7 b	25.9 abc	32.7 ab
K-carb-35™ 4000 ppm K	5.6 a	9.5 ab	NT	NT
K-carb-35™ 2000 ppm K	8.7 a	18.1 b	NT	NT
K-carb-35™ 400 ppm K	NT	NT	8.9 a	40.7 ab
K-carb-35™ 200 ppm K	NT	NT	44.0 bc	58.0 b
K-carb-35™ 50 ppm K	NT	NT	7.4 a	22.6 ab
Silvine® 1000 ppm Si	NT	NT	55.4 c	11.4 a
Silvine® 500 ppm Si	NT	NT	3.0 a	19.4 ab
Silvine® 50 ppm Si	1.4 a	13.3 ab	NT	NT

[1]Values were used for analysis of variance calculations and letters in common represent no significant difference ($P<0.05$).
[2]NT= not tested

In the final pot trial, efficacies of products were evaluated based on germination rates only. This was because disease occurred very early in this trial resulting in very low levels of germination and thus insufficient seedlings were available to evaluate rates of disease development post-emergence. As observed with previous trials, higher levels of disease developed in sterilised soil as compared to the same soil non-sterilised (Table 8.6). Most of the products when used to treat either sterilised or non-sterilised soil failed to significantly enhance disease control as compared to the inoculated controls. The exception was when Stand SKH™ was added to sterilised soil at 500 ppm Si. In this case, disease

control was significantly higher than the inoculated control but still well below the non-inoculated control.

Table 8.6. The efficacy of potassium and silicon products in the control of *P. ultimum* induced disease of beetroot as determined in pot trial 3. Efficacy was evaluated as the percent of seeds germinated.

Treatment	Non-sterilised soil (%)	Sterilised soil (%)
Uninoculated control	86.7 c[1]	93.3 c
Inoculated control	40.0 ab	6.7 a
Kasil 2040® at 50 ppm Si	40.0 ab	6.7 a
Kasil 2040® at 500 ppm Si	53.3 abc	0.0 a
Kasil 2040® at 1000 ppm Si	33.3 ab	0.0 a
Silvine® at 50 ppm Si	53.3 abc	0.0 a
Silvine® at 500 ppm Si	13.3 a	0.0 a
Silvine®at 1000 ppm Si	33.3 ab	0.0 a
K-carb-35™ at 50 ppm K	13.3 a	6.7 a
K-carb-35™ at 500 ppm K	66.7 bc	0.0 a
K-carb-35™ at 1000 ppm K	13.3 a	6.7 a
Stand SKH™ at 50 ppm Si	53.3 abc	20.0 ab
Stand SKH at 500 ppm Si	33.3 ab	40.0 b
Stand SKH at 1000 ppm Si	26.7 ab	6.7 a

[1]Values were used for analysis of variance calculations and letters in common represent no significant difference ($P<0.05$).

In addition to the pot trials for control of *P. ultimum*, the efficacy of the nutrient and SAR products on the control of disease in beans by *P. aphanidermatum* was also evaluated. The first trial used the same experimental conditions as those for the first *P. ultimum* trial used to evaluate the potassium and silicon products. However, the second trial varied as pasteurised potting mix, more inoculum and different rates of the products were used. Additionally, the products were applied three times instead of just once at planting and the average minimum and maximum temperatures were higher, 21°C and 34°C, respectively. The results for these trials are summarised in Table 8.7.

In the first bean trial, germination was relatively uniform in the sterilised soil but quite variable in the non-sterilised soil. In particular, the use of Kasil 2040® and Stand SKH™ both at 5000 ppm Si resulted in significantly lower germination than the control treatments (Table 8.7). When non-sterilised soil was used, no significant differences were observed between the level of seedling disease resulting from use of any product and the inoculated control. By contrast, more disease was observed in seedlings grown in this soil after it was sterilised but no treatments resulted in significantly improved disease control.

In the second trial, product efficacies were evaluated based on germination rates only as disease occurred very early thus insufficient seedlings were available to evaluate rates of seedling disease development. This is probably a result of using a higher rate of inoculum and the warmer temperatures experienced during this trial compared to the previous trial. No products gave significantly improved

disease control in this trial; instead all of products significantly reduced germination rates as compared to the non-inoculated control.

As there was such low germination in some of these trials a negative effect on germination resulting from the use of these products in the absence of *Pythium* can not be discounted and would require testing.

Table 8.7. The efficacy of potassium and silicon products in the control of *P. aphanidermatum* induced disease of beans as determined in pot trials 1 and 2. Efficacy was evaluated as the percent of seeds germinated (G) and as the percent of seedlings affected (A). This was done for non-sterilised (NS), sterilised (S) soil and potting mix (P)

	Trial 1				Trial 2
Treatment	G NS (%)	G S (%)	A NS (%)	A S (%)	G P (%)
Uninoculated control	93.3 b[1]	66.7 a	6.7 a	0.0 a	88.9d
Inoculated control	93.3 b	80.0 a	13.3 a	33.3 ab	55.6 c
Kasil 2040® at 50 ppm Si	NT[2]	NT	NT	NT	16.7 ab
Kasil 2040® at 500 ppm Si	NT	NT	NT	NT	22.2 ab
Kasil 2040® at 1000 ppm Si	NT	NT	NT	NT	22.2 ab
Kasil 2040® at 5000 ppm Si	53.3 a	66.7 a	20.0 a	26.7 ab	NT
Bion® 25 µg/ml	93.3 b	93.3 a	6.7 a	13.3 ab	NT
Stand SKH™ at 50 ppm Si	NT	NT	NT	NT	22.2 ab
Stand SKH™ at 500 ppm Si	NT	NT	NT	NT	38.9 bc
Stand SKH™ at 1000 ppm Si	NT	NT	NT	NT	22.2 ab
Stand SKH™ at 5000 ppm Si	53.3 a	66.7 a	13.3 a	53.3 b	NT
K-carb-35™ at 50 ppm K	NT	NT	NT	NT	11.1 a
K-carb-35™ at 500 ppm K	NT	NT	NT	NT	33.3 abc
K-carb-35™ at 1000 ppm K	NT	NT	NT	NT	22.2 ab
K-carb-35™ at 2000 ppm K	86.7 b	80.0 a	13.3 a	33.3 ab	NT
K-carb-35™ at 4000 ppm K	73.3 ab	73.3 a	26.7 a	40.0 ab	NT
Silvine® at 50 ppm Si	86.7 b	73.3 a	13.3 a	20.0 ab	16.7 ab
Silvine® at 500 ppm Si	NT	NT	NT	NT	27.8 ab
Silvine® at 1000 ppm Si	NT	NT	NT	NT	22.2 ab

[1]Values were used for analysis of variance calculations and letters in common represent no significant difference ($P<0.05$).
[2]NT= not tested

The efficacy of Bion® and potassium and silicon products as seed treatments to control disease of beetroots caused by *P. ultimum* and disease of bean caused by *P. aphanidermatum* was also evaluated in a single trial. No products were identified as having effective disease control. A summary of key points is provided.

The percent germination of bean seeds planted in sterilised soil inoculated with *P. aphanidermatum* was significantly lower than the non-inoculated control for all treatments with the exception of those seeds soaked in Bion®, which gave a similar germination rate to this control. However, although 67 % of the seeds soaked in Bion® germinated, compared to only 13 % of seeds in the inoculated control, most (82 %) of the subsequent developing seedlings succumbed to disease. The percent germination of beetroot seeds planted in sterilised soil inoculated with *P. ultimum* was also very poor. The highest rate (46.7 %) was observed from seeds soaked in 4000 ppm K-carb-35™ but again there was a high (64 %) mortality rate amongst the developing seedlings.

When the soil was used without sterilisation the percent germination of both bean and beetroot seed was very poor in all treatments. Only 13 % to 53 % of bean seed and less than <15 % of beetroot seeds germinated.

8.3.4 Pot trials to evaluate surfactant products

The efficacy of several surfactant products on the control of disease in beetroot by *P. ultimum* was evaluated using two different field soils in separate trials. The details for the three trials are as described for those used to evaluate the nutrient and SAR products and the results are summarised in Table 8.8.

For trial one, no significant difference in germination was observed between products and controls for sterilised soil, but the use of Hortiwett® significantly improved germination compared to the inoculated control in non-sterilised soil (Table 8.8). However, using this product resulted in more Pythium-affected seedlings than the non-inoculated control. None of the other products used to treat either soil type had a significant effect on disease rates, positively or negatively.

The germination rates observed in the second trial were relatively uniform in both sterilised and non-sterilised soil (results not shown). There were no significant differences in the development of disease between any of the products when applied to non-sterilised soil. By contrast, treatment of sterilised soil with Agral® or DuWett®at 20 µg/ml significantly decreased disease rates as compared to the inoculated control whereas the remaining products gave similar disease rates to the inoculated control (Table 8.8).

Table 8.8 The efficacy of surfactant products in the control of *P. ultimum* induced disease of beetroot as determined in pot trials 1 and 2. Efficacy was evaluated as the percent of seeds germinated (G) and as the percent of seedlings affected (A). This was done for non-sterilised (NS) and sterilised (S) soil.

	Trial 1				Trial 2	
Treatment	G NS (%)	G S (%)	A NS (%)	A S (%)	A NS (%)	A S (%)
Uninoculated control	73.3 ab[1]	60.0 ab	3.0 a	2.1 a	0.0 a	0.0 a
Inoculated control	40.0 a	60.0 ab	3.2 a	8.8 ab	18.5 a	43.0 b
Du-Wett® at 20 µg/ml	73.3 ab	46.7 ab	5.6 a	12.8 ab	20.0 a	0.0 a
Du-Wett® at 50 µg/ml	NT	NT	NT	NT	0.0 a	29.9 ab
Hortiwett® at 20 µg/ml	80.0 b	73.3 b	14.3 a	20.0 b	12.2 a	30.7 ab
Hortiwett® at 50 µg/ml	NT	NT	NT	NT	6.7 a	46.5 b
Agral®at 20 µg/ml	73.3 ab	40.0 a	8.3 a	13.3 ab	2.8 a	6.7 a
Agral® at 50 µg/ml	NT	NT	NT	NT	3.3 a	29.6 ab

[1]Values were used for analysis of variance calculations and letters in common represent no significant difference ($P<0.05$).

In the final pot trial, efficacies of products were evaluated based on germination rates only as insufficient seedlings were available to evaluate rates of disease development post-emergence. Again this is probably a result of using a higher rate of inoculum and the warmer temperatures experienced during this trial compared to the previous trial. This trial evaluated the three surfactants at concentrations of 20, 50 and 100 µg/ml. The germination rates in non-sterilised and sterilised soil when treated with any of the products were all significantly lower than the non-inoculated control (results not shown).

In the first pot trial to evaluate the efficacy of the three surfactant products against *P. aphanidermatum,* a concentration of 20 µg/ml was used. In this trial the percent germination was relatively uniform for both non-sterilised and sterilised soil and there were no significant differences in the development of disease between treatments for the non-sterilised soil. By contrast, in the sterilised soil disease developed to higher levels but no products were effective in controlling this disease (results not shown).

The second bean trial included a further two rates (50 and 100 µg/ml) of the three products, used pasteurised potting mix and the application method altered to give multiple exposures to the product. None of the products tests resulted in improved disease control as indicated by the significantly lower germination rates observed for all treatments compared to the non-inoculated control. Instead, Agral®at any of the three rates used and DuWett® when used at 100 µg/ml, resulted in significantly less germination than the inoculated control (Table 8.9).

As there was such low germination in some of these trials a negative effect on germination resulting from the use of these products in the absence of *Pythium* can not be discounted and would require testing.

Table 8.9. The efficacy of surfactant products in the control of *P. aphanidermatum* induced disease of beans as determined in pot trial 2. Efficacy was evaluated as the as the percent of seeds germinated.

Treatment	Germination (%)
Uninoculated control	89.9 d[1]
Inoculated control	55.6 c
Du-Wett® at 20 µg/ml	33.3 abc
Du-Wett® at 50 µg/ml	27.8 abc
Du-Wett® at 100 µg/ml	16.7 ab
Hortiwett® at 20 µg/ml	38.9 bc
Hortiwett® at 50 µg/ml	27.8 abc
Hortiwett® at 100 µg/ml	27.8 abc
Agral® at 20 µg/ml	22.2 ab
Agral® at 50 µg/ml	22.2 ab
Agral® at 100 µg/ml	5.6 a

[1]Values were used for analysis of variance calculations and letters in common represent no significant difference ($P<0.05$).

8.3.5 Phytotoxic effects of test products

Plants were monitored for evidence of phytotoxic effects in all pot trials by visual examination only. The only phytotoxic effect observed was in those trials established to evaluate nutrient and SAR products for disease control. The effect was associated with the use of Bion® at a concentration of 25 µg/ml. In beetroot plants the effect manifested as a reddening of leaves and a reduction in plant growth (Figure 8.1). By contrast, the bean plants remained a normal in colour but exhibited severely deformed leaves (Figure 8.2).

(a)

(b)

(c)

Figure 8.1. Photographs of beetroot plants exhibiting phytotoxicity to Bion® when it was used to drench soil at a rate of 25 µg/ml. In (a) healthy plants are on the far left and right of the photo with those in the three central pots showing signs of phytotoxicity to Bion®. Similarly, plants shown in (b) had an advsere affect to bion compared to the healthy plants from the same pot trial shown in (c). Plants in (a) were grown in a granite-base soil whereas those in (b) and (c) were grown in a clay-based soil.

Figure 8.2. Photograph of bean plants exhibiting phytotoxicity to Bion® when it was used to drench soil at a rate of 25 µg/ml. Healthy plants are shown on the far right of the photo with the remaining plants showing an adverse affect to the Bion®. Plants in were grown in a granite-base soil.

8.4 Discussion

In summary, under moderate to high inoculum levels it is unlikely that any of these products would provide benefit in the control of diseases caused by *P. aphanidermatum* or *P. ultimum* using any of the application methods trialled in this project. Some of the products may provide benefit under low disease pressure or using more refined application methods and/or product concentrations but this would need further investigation.

In general there was a higher level of disease observed in plants grown in sterilised as compared to non-sterilised soil. This could suggest a level of natural suppression of *Pythium* present in the field soils resulting from the presence of other microbes and that the application of the test products at the rates used in these experiments did not reduce the populations of these microbes to levels equivalent to sterilisation. However, autoclaving soil has also been shown to release nutrients and toxins which were previously bound within the soil (Nehl, 1996) and that may negatively affect plant growth causing increased susceptibility to pathogens. The effect of autoclaving on plant susceptibility to *Pythium* was not evaluated.

The incorporation of test biofumigant products into media to evaluate *in vitro* growth of pathogens was not found to be a useful method for determining if those products will be efficacious for control of disease in soil. In this study, the results from pot trial evaluations of volatile oils did not necessarily reflect the results from the *in vitro* assays. In particular, clove oil which was very effective at limiting mycelia growth of Pythium *in vitro* was not useful in control of disease. Using alternative methods for *in vitro* evaluation such as creating volatile chambers (see chapter 5) may provide a better means of rapid screening with results which correlate more readily with applications to soil. However, the efficacy of the products for disease control may be influenced greatly by abiotic and biotic soil factors, making it unlikely that a single product at a particular application rate would work in all soil types.

Treatment of seed with products that provide protection against infection by Pythium prior to planting would be a practical method of disease control. This was briefly investigated in this study through a single pot trial using one seed treatment method. However, the soaking of beetroot and bean seed in the test nutrient and SAR products did not provide useful control of disease induced by *P. ultimum* or *P. aphanidermatum*. Other methods for treating seed such as slurries, osmopriming or solid-matrix-priming were shown to provide control against Pythium for some products, in a few specific host-pathogen combinations (Pill *et al.* 2009; Rush 1994). Thus, further research in this area would be beneficial.

Unless applied as seed treatments, or alternatively as drenches applied in the nursery, the use of nutrient and SAR products to provide adequate control of *Pythium*-induced seedlings disease is questionable. To be utilised properly, the plant requires time to assimilate and process these products into pathogen defence or avoidance pathways, thus post-planting or post-emergence applications will in most cases be too late to provide a useful level of disease control. The results from this study also indicate these products have a very low efficacy for direct inhibition on *Pythium* growth. No inferences could be drawn from this study on the effectiveness of using silicon and potassium alone or as a combined product for control of *Pythium* diseases. However, further research in this area would be beneficial.

In previous studies, surfactants were shown to be effective against *Pythium* spp. through their mode of action on zoospore formation and motility (Stanghellini and Tomlinson 1987) but their effectiveness on non-zoospore producing pathogens is not known. This study compared the efficacy of three surfactants against two species of Pythium, one with high and the other without zoospore production. However, none of the products at the rates tested in this study were consistently effective in either limiting mycelia growth *in vitro* or at controlling disease induced by *P. ultimum* or *P. aphanidermatum*.

8.5 References

Bekker TF, Kaiser C, Labuschagne N (2009) The antifungal activity of potassium silicate and the role of pH against selected plant pathogenic fungi *in vitro*. *South African Journal of Plant Soil* **26**, 55-57.

Bekker TF, Kaiser C, Merwe Rvd, Labuschagne N (2006) *In vitro* inhibition of mycelial growth of several phytopathogenic fungi by soluble potassium silicate. *South African Journal of Plant Soil* **23**, 169-172.

Cherif M, Menzies JG, Ehret DL, Bogdanoff C, Belanger RR (1994) Yield of Cucumber Infected with Pythium aphanidermatum when Grown with Soluble Silicon. *Hortscience* **29**, 896-897.

Datnoff LE, Elmer WH, Huber DM (2007) (Ed.)^(Eds) 'Mineral nutrition and plant disease.' (The American Phytopathological Society: St Paul, Minnesota)

Mondal AH, Nehl DB, Allen SJ (2005) Acibenzolar-S-methyl induces systemic resistance in cotton against black root rot caused by *Thielaviopsis basicola*. *Australasian Plant Pathology* **34**, 499-507.

Nehl DB (1996) The role of arbuscular mycorrhizal fungi, rhizobacteria and properties of the soil in a growth disorder of cotton., University of New England.

Nehl DB, Allen SJ, Brown JF (1996) Deleterious rhizosphere bacteria: an integrating perspective. *Applied soil ecology* **5**, pp. 1–20.

Pill WG, Collins CM, Goldberger B, Gregory N (2009) Responses of non-primed or primed seeds of 'Marketmore 76' cucumber (Cucumis sativus L.) slurry coated with Trichoderma species to planting in growth media infested with Pythium aphanidermatum. *Scientia Horticulturae* **121**, 54-62.

Rush CM (1994) Comparison of seed priming techniques with regard to seedling emergence and pythium damping-off in sugar beet. *Phytopathology* **81**, 878-882.

Stanghellini ME, Tomlinson JA (1987) Inhibitory and lytic effects of a nonionic surfactant on various asexual stages in the life cycle of *Pythium* and *Phytophthora* species. *Phytopathology* **77**, 112-114.

Tomlinson JA, Faithfull EM (1980) Effects of fungicides and surfactants on the zoospores of *Olpidium brassicae*. *Annals of Applied Biology* **93,** 13-19.

Vallad GE, Goodman RM (2004) Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science* **44**, 1920-1934.

Van der Plaats-Niterink J (1981) Monograph of the genus Pythium. *Studies in Mycology* **21**, 1-240.

Technology Transfer

9. Technology Transfer

Researchers: Entire project team

This chapter details the communication activities and outcomes for the project. It has been prepared using the original project communication plan as the backbone against which the activities and outcomes are assessed. A range of communication strategies were employed including workshops, field days, science forums, presentations and displays at industry conferences and events, industry articles and scientific papers. Of these the most effective were on-farm field days, roadshow style workshops and a display at the national vegetable expo.

9.1 Key Communication Activities

1) Roadshow/workshops

Initial roadshow activities including workshops and farm visits to communicate world's best practice IPM strategies for managing soilborne diseases were held during 2008. Activities were regionally focused and visiting scientists were selected based upon their expertise with the key pathogen problems in each region. For example, in the Northern Territory the focus was on Fusarium wilt, in Queensland Sclerotinia and in Victoria soilborne pathogens in general were covered. Ross Holding, a consultant working with Peter dal Santo on the chemical best practice program (VG07109) presented up to date information on chemicals registered and available for use under permit in each state for the pathogens of interest, together with some 'best practice' use and 'IPM fit' information. The following workshops were held:
Granite Belt (QLD) – 1st July 2008 (7 in attendance)
Lockyer Valley (QLD) – 2nd July 2008 (11 in attendance)
Cranbourne (VIC) – 3rd July 2008 (13 in attendance)
Darwin(NT) – 23rd/24th September 2008 (21 in attendance), followed by visit to 5 farms.

A second series of roadshow events was held during the second half of 2010. Activities were again regionally focused and visiting scientists were selected based upon their expertise with key pathogen problems and issues of interest in each region. At each workshop attendees were provided with a pack containing workshop notes. All workshops were well attended (20-50 attendees per event) and feedback was extremely positive. Details are provided below.

Devonport (TAS) – 4th August 2010 (34 in attendance)
Topics : Sclerotinia control, biofumigation/green manures, soil health, novel/developing soilborne disease management tools, disease prediction, managing downy and powdery mildew, white blister and anthracnose.
Speakers: Ian Porter, Caroline Donald, Oscar Villalta, Liz Minchinton (DPI Victoria). Hoong Pung (Peracto TAS), Susie Jones (UTAS).

Gympie (QLD) – 11th August 2010 (34 in attendance)
Topics : Sclerotinia control, biofumigation/green manures, soil health, novel/developing soilborne disease management tools, disease prediction, managing downy and powdery mildew, white blister and anthracnose, viruses in vegetable crops.
Speakers: Ian Porter, Caroline Donald, Oscar Villalta, Liz Minchinton, Joanna Petkowski (DPI Victoria). Elio Jovicich, Denis Persley (DEEDI QLD)

Gatton (QLD) – 12th August 2010 (46 in attendance)

← Image previous page, chapter 9 coversheet – growers inspecting plots, Clyde VIC field day 2009.

Topics : Sclerotinia control, biofumigation/green manures, soil health, novel/developing soilborne disease management tools, disease prediction, managing downy and powdery mildew, white blister and anthracnose, viruses in vegetable crops.
Speakers: Ian Porter, Caroline Donald, Oscar Villalta, Liz Minchinton, Joanna Petkowski (DPI Victoria). Elio Jovicich, Denis Persley (DEEDI QLD)

Lindenow (VIC) – 18th August 2010 (20 in attendance)
Topics : Sclerotinia control, biofumigation/green manures, soil health, novel/developing soilborne disease management tools, disease prediction, managing downy and powdery mildew, white blister and anthracnose.
Speakers: Caroline Donald, Oscar Villalta, Liz Minchinton, Joanna Petkowski, Cassie Scoble (DPI Victoria).

Cranbourne (VIC) – 19th August 2010 (23 in attendance)
Topics : Sclerotinia control, biofumigation/green manures, soil health, novel/developing soilborne disease management tools, disease prediction, managing downy and powdery mildew, white blister and anthracnose.
Speakers: Caroline Donald, Oscar Villalta, Liz Minchinton, Joanna Petkowski, Cassie Scoble (DPI Victoria).

Darwin (NT) – 25th November 2010 (50 in attendance)
Topics: Soil cultivation with particular reference to hard pans, use of mulches, green manures, snake bean grafting and post-graft care, root knot nematode, use of registered chemicals, insects of vegetable crops, cucurbit mosaic viruses. This event featured practical in-field demonstrations and hands-on exercises, attendees received a DVD on grafting snake bean in addition to the workshop pack.

Speakers: Stuart Smith, Les Huth, Barry Condè, Sean Bithell, Mark Hoult, Peter Bergin, Brian Thistleton (Plant Industries, NT Department of Resources), Geoff Walduck (Biosecurity and Product Integrity, NT Department of Resources).

Bathurst (NSW) – 9th December 2010 (20 in attendance)
Topics : Biofumigation/green manures, soil health, novel/developing soilborne disease management tools, disease prediction, managing downy and powdery mildew, white blister and anthracnose, general questions and answers.
Speakers: Ian Porter, Caroline Donald, Liz Minchinton (DPI Victoria). Len Tesoriero (NSW I & I).

2) Science Forums

Three workshops were held at the beginning of the project (late 2007). These pathogen specific science forums brought together expert pathologists, chemical, vegetable and processing industry representatives to review current control strategies and prioritise further research. Information gained from these meetings, together with a review of the available literature was used to produce a review 'Towards sustainable integrated control of soilborne plant diseases - A review of available and emerging chemical and non-chemical strategies and their potential compatibility with IPM programs'. This document presented to the IAC early in 2008 was used to plan specific research modules of the project. The project team is grateful to all who shared their expertise through their participation in the workshops.

Sclerotinia workshop (Tasmania)
Oscar Villalta (DPI Vic), Denise Wite (DPI Vic), Calum Wilson (UTAS), John Duff (QDPI & F), Garry McNab (Simplot), Sue Cross (Bayer), Doug Wilson (NuFarm), Rob Velthius (Xeron), Ian Porter (DPI Vic) , Caroline Donald (DPI Vic), Barry Kerr (Sumitomo), Alicia Greenhill (La Trobe Uni), Rebecca Barnett (La Trobe Uni), Kim Plummer (La Trobe Uni), Hoong Pung (Peracto), Suzie Jones (UTAS), Frank Hay(TIAR/UTAS), Sarah Pethybridge (TIAR/UTAS), David Gent(USA) , Philip Frost (Peracto).

Pythium & Fusarium workshop (Sydney)
Graham Stirling (Biological Crop Protection), David Nehl (NSW DPI), Christine Horlock (QLD DPI & F), Edward Leiw (Botanic Gardens) , Leanne Forsyth (NSW DPI), Liz Minchinton (VIC DPI), Andrew Watson (NSW DPI), Ameera Youseph (Syd Uni), Des Auer (VIC DPI), Luc Streit (Syngenta), Rob Velthius (Xeron), Len Tesoriero (NSW DPI), Ian Porter (VIC DPI), Caroline Donald (VIC DPI), Barry Conde (NT DPIFM), Barbara Hall (SARDI), Trevor Klein(Syngenta), Peter Dal Santo (Agaware).

Rhizoctonia workshop (Melbourne)
Dolf deBoer (DPI VIC), Jon McCarthy (BarMac), Susan Cross (Bayer), Alistar Beyer (Bayer), Peter dal Santo (Agaware), Rob Velthuis (Xeron), Scott Mattner (DPI), Ken Labbett (PPIAC), Iain Kirkwood (PPRD), Chris Russell (Simplot), James Hills (Agronico), Mark Atkinson (Syngenta), Trevor Stebbings (Syngenta), Hoong Pung (Peracto), Chris Van der Hoven (Sumitomo), Barry Ker (Sumitomo), Andrew Watson (Agnova Technologies), Ian Fraser, Michael Tool (Yield Enhancement Services), Kathy Ophel-Keller (SARDI), Neville Fernando (DPI), Barbara Hall (SARDI), Cathy Todd (Uni Adelaide), Ian Porter (DPI), Cassie Scoble (DPI), Andrew Pittman (Crop and Food NZ), Peter Lane (Lane Bros Warnambool VIC), Caroline Donald (DPI), Luc Streit (Syngenta), Phil Jobling.

3) Farm walks/grower field days

Farm walks and grower field days were held during years 2 and 3 of the project to demonstrate IPM strategies in key regions.

The project team were instrumental in organising the IPM vegetable diseases display at the National Vegetable Expo. The team prepared poster and project flyer templates which were used by the entire vegetable pathology group. Hands on displays were created and members of the project team staffed the marquee discussing the displays and information available with hundreds of growers attending the expo.

In Victoria farm walks/grower field days demonstrated the use of biofumigant and green manure break crops to reduce soilborne inoculum and disrupt the infection process. High glucosinolate brassica crops (mustards) together with a range of green manure crops from different plant families (cereals - oats, triticale and ryecorn, legumes - faba beans and vetch, grasses - ryegrass together with fallow) were grown in replicated field plots. Field days were held at two trial sites in different regions when the mustard crops reached full flowering, the point at which the content of biofumigant compounds in the plant tissue is maximum. Both Victorian field days were held in conjunction with HAL program 2.1, the Sclerotinia subprogram. Both events were well attended (approx 20-25 at each event). Ian Porter presented the background to the IPM disease program and the aims of subprograms 2.1 and 2.2 were presented. Growers were then invited to walk the plots with researchers and to discuss crop growth and the advantages and any potential risks associated with each crop type. A range of brief presentations followed. Oscar Villalta presented an update on Sclerotinia control. Caroline Donald introduced a range of novel techniques currently being evaluated for the control of soilborne diseases. Scott Mattner gave a practical demonstration of biofumigant activity and the techniques used to measure isothiocyanates (ITCs) in soil following incorporation of mustard crops. Each field day was concluded with a machinery demonstration to illustrate the pulverising technique required mulch the plant tissue sufficiently to release the volatile compounds to the soil.

In the Northern Territory four field trials were conducted to demonstrate the use of grafting to control Fusarium wilt in snake beans. Grafting exploits genetic resistance to Fusarium wilt that is found in Iron cowpea. Snake bean seedlings are grafted onto the resistant Iron cowpea root stock. The resistant root stock protects the susceptible snake bean from *Fusarium* wilt thereby strengthening this host crop against disease. Two field days were held. At the first site seedling snake beans had 98.8 % infection (83/84) compared with no infection in the grafted plants at the time of the field day. The Fusarium wilt infection was so severe that the non-grafted

seedling plants failed to produce any harvestable beans. Growers involved in the trial came, as well as three staff from Plant Pathology and the acting Director Plant Industries, NT DRDPIFR. A second field day was held on a second farm on Wednesday 5th August. Seedling snake beans had 96.67 % infection (116/120) compared with no infection in the grafted plants at the time of the field day. All growers at the second field day were "new farmers", not yet in the project team's group of participating growers. They are interested in the grafting technology because they have a severe *Fusarium* wilt problem in their snake bean crops. All these growers have been given seed of the Iron cowpea rootstock to multiply for further trials on their properties. The farmers at the second field day not only inspected the trial, but also eagerly looked at the farmer's latest newly planted grafted plants and also the oldest planting of grafted plants. They were very impressed that the farmer had grown over 4000 grafted plants in the previous year, and that the life of the first grafted planting is being extended from five weeks to approximately four months in response to additional fertilizer.

The following farm walks/grower field days have been held:
National Vegetable Expo, Werribee, Victoria 7-8th May 2009
Lindenow, Victoria 10th June 2009
Clyde, Victoria 19th June 2009
Berry Springs, Northern Territory 15th July 2009
Acacia Hills, Northern Territory 5th August 2009
Darwin, Northern Territory 25th November 2010

4) Industry articles

Vegetables Australia editor (Jim Thompson) was approached and asked to consider publishing regular subprogram updates in Vegetables Australia. He advised that the magazine would not accept regular updates but would consider a one off article. An article was prepared in conjunction with program 2.1 focusing on Sclerotinia, the most important soilborne pathogen affecting vegetable crops. 'Smart strategy beats cabbage-patch killer' Vegetables Australia, Vol 4.6 May / June 2009, pp42-43.

An article 'Year of the snake' highlighting the problem of Fusarium wilt in snake beans and the use of grafting to manage it was prepared and published in Territory Quarterly, third quarter 09 p56-57.

In addition two project flyers were prepared. The first of these was used in workshop booklets handed out to participants in project roadshow activities held early in the project. This flyer was updated and subsequently used in the IPM Vegetable Diseases Program overview booklet which was a key means of providing 'take home' information to attendees at the Vegetable Industry conference, National Vegetable Expo and project farm walks/grower field days.

5) Best practice guide/flyer/brochures

As the project progressed it became evident that, with the exception of *Sclerotinia*, the key soilborne diseases being targeted by the project are predominantly found as disease complexes (*Pythium, Fusarium* and *Rhizoctonia* spp.). Very rarely do these pathogens act alone to cause root or stem rotting symptoms. With the exception of *Sclerotinia* therefore the concept of a best practice for these individual pathogens becomes meaningless. As the project has progressed research has been directed towards developing general IPM practices and novel disease control strategies that will reduce the effects of soilborne diseases in vegetable crops in general rather than focus on any individual host-pathogen target. 'Best practice guides' were prepared and distributed as a package at the 2010 roadshow events. The guides were prepared collaboratively across a number of projects within the vegetable pathology program:
Managing Soilborne Diseases
Managing Sclerotinia (with project VG07126)
Managing Mildews, Anthracnose and White Blister (with project VG07070)
Improving soil health (with project VG07008)
In addition, growers attending the Darwin 2010 roadshow event received an Agnote prepared by the Northern Territory project team 'Grafting snake beans to control Fusarium wilt' (Conde *et al.* 2010) and a DVD on grafting snake beans.

6) Other communication activities

Oral presentations at conferences or industry events

- 'Project overview' at the Vegetable Industry Conference Melbourne, 6th May 2009 (presenter C. Donald & O. Villalta).
- 'Novel biological methods for crop protection against soilborne pathogens derived from endophytic fungi, Australasian Soilborne Diseases Symposium, Thredbo, NSW 5-7th Feb 2009 (presenter R. Mann).
- 'Evaluation of essential oils and other plant extracts for control of soilborne pathogens of vegetable crops. Australasian Plant pathology conference, Newcastle, NSW 29 Sept-1 Oct 2009 (presenter C. Scoble).
- 'The potential of biofumigant and green manure crops as a tool to manage soilborne diseases in vegetable production' 6th Australasian soilborne Diseases Symposium, Twin Waters, QLD 9-11th August 2010 (presenter C. Donald).
- 'Sustainable management of soilborne diseases'. Horticultural Industry Network, Packenham, VIC 2nd December 2010 (presenter C. Donald).

Conference presentations (posters)

- 'T-DNA insertion mutagenesis to identify sclerotial development genes in Sclerotinia sclerotiorum 2009 Fungal Genetics conference California USA (presenter A. Greenhill)
- 'Project overview' poster used at the Vegetable Industry Conference Melbourne, 6th May 2009 and National Vegetable Expo 7-8th May 2009 (presenter C. Donald & O. Villalta).
- 'Effect of plant extracts on mycelial growth of soiborne pathogens causing root rot and wilt of vegetables'. 6th Australasian Soilborne diseases symposium, Twin Waters, QLD 9-11th August 2010 (presenter C. Scoble).

Program meetings

Program meetings were held annually. Project leaders attended. These were extremely useful planning tools. These were supplemented during the second half of the project with program conference calls (implemented by program leader Sarah Sullivan) on an as needs basis. These calls facilitated by the program leader were an excellent means of keeping everyone informed and getting rapid consensus from the program team on a current issue.

Other industry articles, flyers and media coverage

- Preparation of a 1 page subprogram summary for the 'IPM Diseases Program' overview booklet and posters used at the industry conference, expo and ongoing communication events.
- Soilborne disease workshop booklet distributed to growers at roadshow workshops during 2008.
- 'Year of the snake'. An article highlighting the problem of Fusarium wilt in snake beans and the use of grafting to manage it. McCue S (2009) "Year of the Snake", Territory Quarterly, Third Quarter 2009: pp56-57.
- 'Smart strategy beats cabbage-patch killer'. Program overview article (programs 2.1 and 2.2). In Vegetables Australia, Vol 4.6 May / June 2009, pp42-43. (written by Oscar Villalta *et al*).
- The ABC Country Hour attended the Darwin roadshow event, broadcasting interviews from participants in the midday program of the 25th November 2010.
- 'Pest control methods examined'. In Bairnsdale Advertiser, 23rd August 2010. (written by journalist attending the Lindenow roadshow workshop 2010).

- 'Latest findings of national IPM disease program unveiled'. In Vegetables Victoria, Issue 42 Spring 2010. (written by Slobodan Vujovic, Victorian IDO after attending Victorian roadshow workshop events 2010).

7) Participation in pathology program communication activities

A communication officer for the IPM Vegetable Diseases program was not appointed duiring the life of the project. Instead, the project team, together with the program manager made every effort to ensure that communication activities were conducted on a 'program' rather than 'project' basis. To this end a program booklet was produced, together with a set of posters. Both of these materials were used to promote the program rather than individual projects at industry events. In addition a large marquee was organised and a program display held at the national vegetable expo (7-8th May, 2009). The marquee was staffed by team members from four states representing many of the projects within the program.

Members of other projects were invited to participate in communication events where relevant to the issues facing the industry in the region where the event was being held.

- Ross Holding (consultant – Classy Solutions) accompanied the project team on all roadshow events to present the most up to date chemical registration and best practice chemical use information.
- Len Tesoriero spoke on Fusarium at the Darwin workshop, 2008 and conducted an open question and answer session at the Bathurst NSW workshop 2010.
- Oscar Villalta and Denise Wite (project 2.1 *Sclerotinia*) accompanied the team to Queensland to participate in 2 workshops and farm visits there during 2008.
- The Clyde Victoria field day 2009 was held in conjunction with a White blister and *Pythium* workshop. Growers moved as a group from the field day to the workshop.
- The temperate vegetable soil health team made presentations and demonstrate the soil carbon calculator at Victorian field days during 2009 and participated in roadshow workshops during 2010.
- Denis Persley (program 5 Virus) and Elio Jovicich (program 3 airborne) presented at roadshow workshops in Gympie and Gatton QLD during 2010.
- Hoong Pung (Peracto) and Suzie Jones (UTAS) both members of project 2.1 *Sclerotinia* presented at the Devonport workshop in TAS during 2010.

8) Scientific papers

The following scientific papers have been prepared during the life of the project. Progress towards publication is given in brackets.

Donald C, Porter I (2009) Integrated control of clubroot. *Journal of Plant Growth Regulation* **28,** 289-303. (published).

Scoble CA (2010) Evaluation of essential oils and other plant derived products as novel potential controls of soilborne pathogens of vegetable crops in Australia. Honours thesis. Botany Department, La Trobe University. 122pp. (published)

Scoble CA, Plummer K, Porter IJ, Donald EC. Inhibitory and biocidal effects of plant extracts on mycelium of soilborne pathogens causing root rot and wilt diseases of vegetables (reviewed, ready for submission).

Donald C, Horlock C, Conde B, Villalta O, Porter I. Towards sustainable integrated control of soilborne diseases. A review of available and emerging chemical and non-chemical strategies and their potential compatibility with IPM programs (reviewed, requested amendments being made).

Scoble CA, Villalta ON, Wite D, Riches D, Porter IJ, Plummer K, Donald EC. Evaluation of plant volatiles as pre-plant soil treatments for control of soilborne pathogens causing vegetable diseases (currently under review).

In addition it is anticipated that the grafting review (part of chapter 4) will be submitted for publication. The biofumigant and green manure research contained in chapter 2 and the *Fusarium* characterisation studies contained in chapter 4 will also be prepared for publication in scientific journals.

9.2 Evaluation of Communication Activities

(See also Appendix 9.1)

General comments

Over the life of the project growers have demonstrated a keen interest in IPM for soilborne diseases. In particular, interest in the use of biofumigant and green manure crops was demonstrated at the vegetable expo and subsequent Victorian field days. Several growers have begun to seek out sources of mustard and other green manure seed to put in their own 'trial' blocks.

There has been good positive feedback from growers and industry representatives. In particular the program booklet which provided short summaries of all of the projects within the program was popular and well received. All attempts to utilise a program approach to communication and extension has been appreciated by growers for whom spare time to attend individual project activities is limited.

Evaluation forms were completed by attendees at each of the 2010 roadshow workshops (excluding Darwin). These forms allowed growers to provide specific feedback on the speakers and topics covered as well as the program in general. The response was overwhelmingly positive with between 94 and 100 % of attendees at each event rating the program as worthwhile (Fig. 9.1).

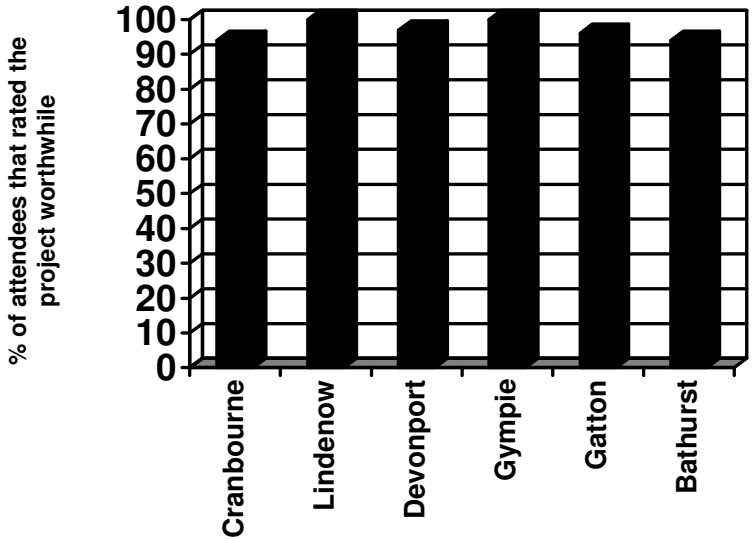


Figure 9.1. Percentage of roadshow workshop attendees that answered yes to the following question: 'Do you rate this IPM program as worthwhile?' Note: of all the attendees only one answered no to this question, the rest were either unsure or failed to answer the question.

Some examples of feedback from local growers

"*We have already seen some positive results on field trials at our property and would like to see further work carried out so that we do not have to rely soley on chemicals*" (Vegetable grower, Victoria).

"I *was worried that with the IDOs going things would fall in a heap but this has really restored my confidence"* (Vegetable grower, attending Victorian field day 2009).

"*If it wasn't for grafting I wouldn't be growing snake beans" and "I wouldn't grow a non-grated plant again unless I was forced to*" (Snake bean grower, Darwin field day 2009).

"Really great – need more of these" (Vegetable grower, Gympie roadshow workshop 2010)

"Excellent workshop – good snap shot of all the work" (Vegetable grower, Gatton roadshow workshop 2010)

"Just keep doing what you're doing" (Vegetable grower, Bathurst roadshow workshop 2010)

Further evaluation

Some comments against individual communication activities have been provided in Appendix 9.1.
Pictorial technology transfer highlights are provided in Appendix 9.2.

9.3 Technology transfer recommendations

This project was a foundation project in the IPM vegetable diseases program. The conclusion of this project and others that ran alongside it within the program creates an ideal opportunity to reflect on the successes of the program and review what has been learned from the communication and extension activities that were part of this project. General observations and recommendations from our communication experiences follow:

1. Program approach has been extremely beneficial and should be encouraged as we enter phase II. This approach has brought together researchers from all over Australia. It has created opportunity for interaction, critical discussions and planning. It has also provided a focus for extension activities. Without a communication officer or IPM coordinator our attempts at coordinated program extension have been restricted. None the less we were able to present an impressive united front at the vegetable industry conference and Vegetable expo which was appreciated by those in attendance. Growers have limited time available to them and we have found that they appreciate any attempts to coordinate delivery of information.
2. Although most workshops, particularly those conducted towards the end of the project (2010) were well attended and provoked useful discussion and interaction, field days with associated practical demonstrations provided greater opportunity for delivery of information in a way that will be understood and remembered.
3. Hands on participation is important for understanding. This project has utilised grafting demonstrations, in field machinery demonstrations, in field 'bucket' experiments and static displays as part of field days and workshops. In all cases, the more hands on the activity, the more discussion is generated and the greater the understanding of the concepts at the end of the day. Simple activities such as getting growers to try grafting, allowing them to sniff volatiles or squeeze the juice from pulverised material after the tractor has passed over it are all keys to memory and improved understanding.
4. Simple one page take home summaries enable participants at communication events to relax and enjoy the activities without feeling the need to take notes. The IPM vegetable diseases program booklet which contained simple 1 page summaries for all projects was an excellent tool for this purpose. This should be updated perhaps every 2 years so that a current version is always available for use.
5. IPM should be developed as a whole farm concept. This should include insect, foliar and soilborne pathology IPM specialists. As researchers we have discovered that opening ourselves up through participation by other research groups in 'our' extension events has led to a much greater understanding of the broader issues facing growers trying to implement IPM. A positive outcome from this approach has been the links established with Victorian insect IPM specialists through their visits to pathology trials. This has resulted in the development of a Phase II project proposal that seeks to develop IPM packages for soilborne diseases that compliment insect IPM strategies. This is the first time that such a coordinated approach has been taken

Appendix 9.1: Communication activities and evaluation
Problem or research focus: Management of key soilborne pathogens (*Sclerotinia, Pythium, Fusarium* and *Rhizoctonia*)
Goal: To develop and evaluate 'best bet' IPM based approaches for the management of *Sclerotinia, Pythium, Fusarium* and *Rhizoctonia*
Intended impact: Increased awareness and adoption of IPM based approaches for the management of *Sclerotinia, Pythium, Fusarium* and *Rhizoctonia*

Who **(Target)**	Type of engagement/how **(Method)**	**Timing**	Monitoring and Evaluation (engagement & impact) **(Measure)**	*Evaluation comments*
Vegetable Industry	1. Roadshow/workshop events 2. Farm walks/grower field days 3. Industry articles 4. Best practice guidelines/flyers/brochures	At least three during first year and ongoing thereafter *(complete)* Years 2 & 3 *(complete)* June, 2008 and twice yearly thereafter *(ongoing – see note)* May-July, 2010. *(outstanding but on track)*	• Regularity of communication; level of feedback received; suggested directions enacted upon • Post-event survey of applicability and understanding; level of discussion generated; qualitative comments from attendees • Timeliness of submission; level of acceptance; qualitative feedback received	*Several events in particular attracted strong positive feedback:* • *Farm walks/grower field days in Victoria – most growers in each region attended. Growers preferred this format to formal workshops and enjoyed the hands on activities.* • *Program booklet distributed at the industry conference, vegetable expo and field days was very well received with many positive comments. Growers appreciated the brief summaries on each project and having everything together in one booklet.* • *Vegetable expo – growers appreciated coordinated approach.* • *Darwin workshop – this event was well attended. Vietnamese growers appreciated having translator in attendance and the hands on grafting demonstrations. This format and the neutral venue on the research farm better suited these growers than field days as there were issues amongst the community regarding family histories and issues of access to old adverseries properties.*
Other Industry sectors (eg. Chemical, fertiliser, non-chemical agriculture industry suppliers, consultants)	1. 1,2,3 & 4 as above. 2. Science forums	As above *(see 1-4 above)* Three by December, 2007 *(complete)*	• As above • Acceptance of research plan for each of the four project modules by IAC.	• *As above* • *In general industry (and growers) appreciated all attempts to present a program approach. With only limited time to participate in communication activities, combined events were well attended.* • *IAC accepted the research plan as presented a the onset of the project.*
Horticulture Australia Ltd	1. Direct communication 2. Milestone reports	On-going As per research agreement schedule 1. *(complete)*	• Regularity of communication; level of feedback received; suggested directions enacted upon • Timeliness of submission; level of	• *All milestones were submitted on time and accepted as submitted with no further information requested.* • *Project staff worked closely with program managers (past and current) and were instrumental in getting program booklets, posters and the Vegetable Expo displays prepared.*

	3. Report to IAC.	May 31st, 2008 *(complete)*	acceptance; qualitative feedback received • Subprogram approved to continue beyond stop-go milestone.	• *The subprogram was approved to continue beyond stop-go milestone.*
Collaborating state Departments of Primary Industries	1. Direct communication 2. Milestone reports	On-going As per research agreement schedule 1. *(complete)*	• Regularity of communication; level of feedback received; suggested directions enacted upon • Timeliness of submission; level of acceptance; qualitative feedback received	• *All milestones and 6 monthly DPI progress reports were submitted on time and accepted as submitted with no further information requested.* • *Feedback from the portfolio manager (BRD) has been positive.*
Wider scientific community nationally and internationally	1. Conference presentations 2. Articles submitted for review in scientific journals	At least once annually *(complete)* At least one annually *(one published, one prepared – ongoing)*	• Formal (during question time) and informal (post presentation) suggestions enacted upon. Issues raised followed up, research plan amended (if required). • Submitted articles published.	• *Presentation at the vegetable industry conference was an excellent means of project exposure with good discussions with potential collaborating growers ensuing. Presentation of plant volatile work by Cassie Scoble led to a developing collaborative relationship with Lincoln University and the offer of a significant VC for future work. Presentation of novel biological methods for crop protection by endophytic fungi by Ross Mann attracted significant interest at the Australasian soilborne diseases symposium. Discussions ensued about the likely rates of use of potential treatments and the most cost effective method of producing endopytes for commercial purposes.* • *The one article submitted was accepted without change. One thesis was published after minor revision. Three papers are at various stages through the review process (see list point 8, section 9.1)* • *There is a 3 month gap between the end of the current project and the beginning of Phase II (if approved). It is intended that this time will be dedicated to preparation of scientific papers arising from work conducted during the life of the project.*

Appendix 9.2 – Technology Transfer Highlights

Mark Traynor (seated) demonstrating grafting technique to NT snake bean growers 23rd Sept 2008.

Grower/industry workshop – Berrimah, NT 23rd Sept 2008.

First tractor pass with the mulcher to demonstrate pulverising the above ground plant tissue, Lindenow VIC, 10 June 2009.

Scott Mattner demonstrating techniques to measure volatile production from biofumigant crops, Lindenow VIC, 10 June 2009.

In-field presentations/demonstrations at the marquee, Clyde VIC, 19th June 2009.

Growers on plots discussing correct equipment and method for pulverisation and incorporation of biofumigant crops, Clyde VIC, 19th June 2009.

Berry Springs NT field day with alternating rows of grafted (healthy) and non-grafted (dead) plants, 15th July 2009.

Acacia Hills NT field day with alternating rows of grafted (healthy) and non-grafted (dead) plants, 5th August 2009.

Growers looking at new plantings of grafted seedlings at the Acacia Hills NT field day, 5th August 2009.

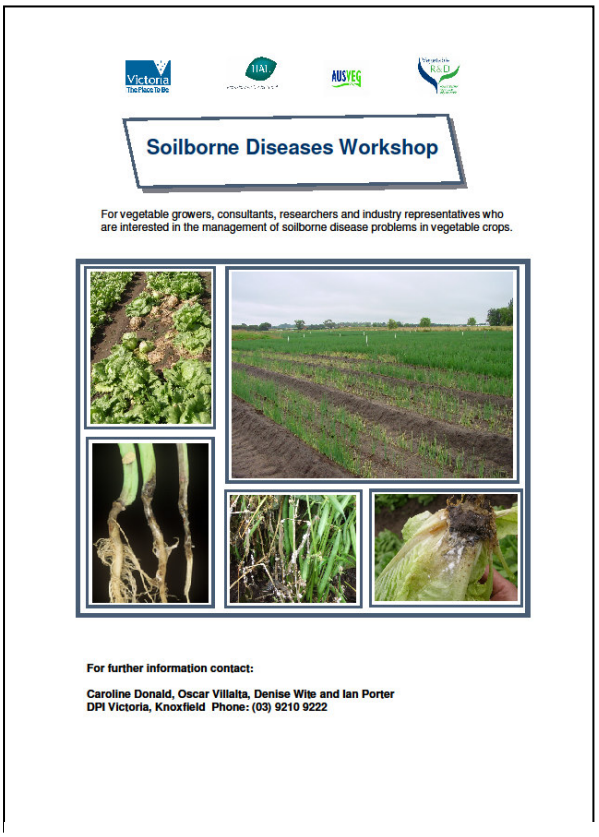
Project team members Denise Wite and Caroline Donald together with members of other HAL IPM disease program projects at the National Vegetable Expo, Werribee VIC, 8th May 2009.

Growers participating in a tillage demonstration as part of the Darwin roadshow workshop/field day 25th November 2010

Growers learn grafting technique and post-graft care as part of the Darwin roadshow workshop/field day 25th November 2010

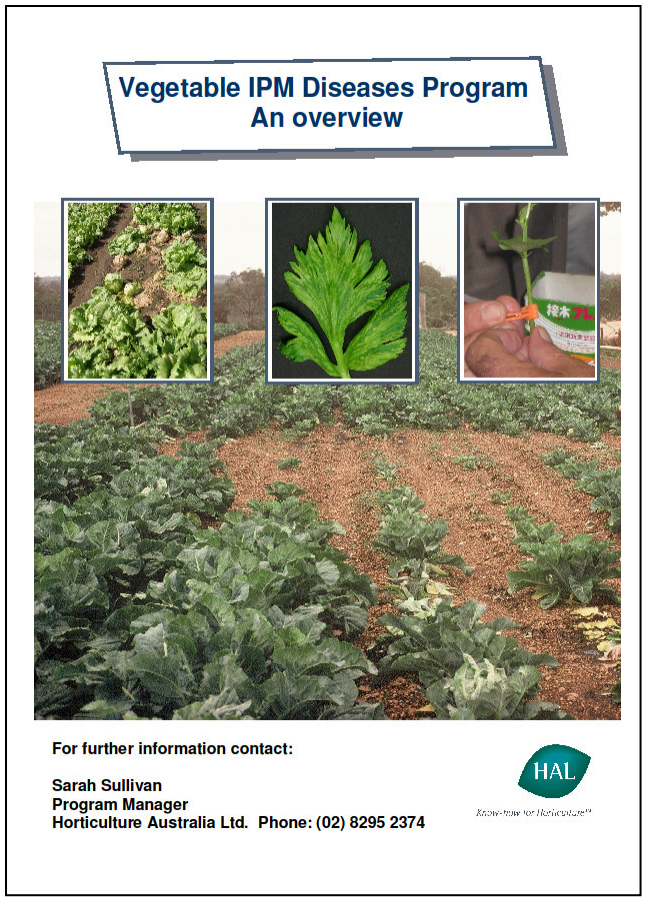
.

Green manure in-field demonstration conducted as part of the Darwin roadshow workshop/field day 25th November 2010

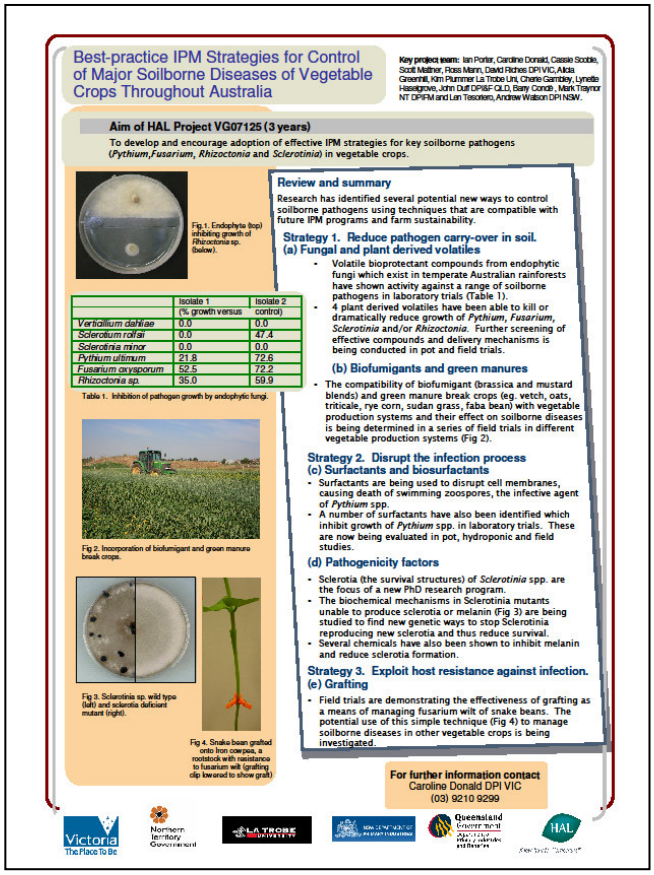


Front cover of workshop booklets used in year 1 roadshow events, 2008.

Growers visiting project displays at the National Vegetable Expo, Werribee VIC, 8th May 2009.



Program overview booklet developed for use at National Vegetable Expo and Vegetable Industry Conference May 2009.



Project summary page from within program overview booklet.