The following organisations sponsored this symposium and the Organising Committee and delegates thank them sincerely for their support.

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**Know-how for Horticulture™**

This conference has been funded by Horticulture Australia Limited using a voluntary contribution from the Organising Committee of the Sixth Australasian Soilborne Diseases Symposium and matched funds from the Federal Government.

**Brisbane City Council**

is proud to support research into soil enhancement through the application of compost and biochar in a south-east Queensland context.

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Remote Microscopy: Protecting biosecurity through diagnostics and training

Remote Microscopy (RM) was developed in response to the biosecurity threats posed by the Emergency Plant Pests (EPPs) and other pests and pathogens. RM overcomes the time and distance that exists between these threats and the experts that identify them to provide a valuable interactive diagnostic communication tool.

RM is unique in that it is a web-based real time diagnostic tool that allows non-experts to rapidly and easily collaborate with experts to identify pest specimens instantly, and so save money and resources. Rapid identification, particularly of exotic pests, is critical to biosecurity response and consequent incursion management.

Centred around Nikon web-based digital cameras and consoles, RM provides a real-time, affordable, widely accessible tool that connects experts and specimens, regardless of location.

The development of the RM network, which is a Cooperative Research Centre for National Plant Biosecurity initiative, is expanding rapidly. It currently extends throughout Australia and New Zealand, Thailand, Lao PDR, Vietnam and East Timor for a total of 41 sites. There are an additional 12 locations Australia wide within the Australian Quarantine and Inspection Service (AQIS).

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Agri-Science Queensland at the Department of Employment, Economic Development and Innovation works to ensure the economic and environmental sustainability of Queensland’s primary industries. Our skilled staff create innovative solutions to pest and disease management through world-leading science and by working with industry to implement effective application of practices.

The Plant Science and Horticulture and Forestry Science groups are investigating management of soil borne diseases in winter cereals, summer field crops and horticultural crops.

Some of the key research areas include developing germplasm with enhanced resistance to crown rot and root lesion nematodes of wheat, investigating reduction of fumigation by rotational cropping sequences, developing rapid screening for resistance to white mould and black rot in peanuts and management of Phytophthora root rot in avocado and pineapple via selection of resistant varieties, and optimising traditional treatments.

For more information on Agri-Science Queensland and our work in soil borne diseases, visit www.deedi.qld.gov.au or call 13 25 23.
PROCEDINGS OF THE SIXTH AUSTRALASIAN SOILBORNE DISEASES SYMPOSIUM

9 - 11 August 2010

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Proceedings, Sixth Australasian Soilborne Diseases Symposium, 9-11 August 2010
(Ed. GR Stirling)

Cover photograph
*Pythium myriotylum* causing rhizome rot of ginger at Eumundi, about 20 km from the conference venue
Welcome to the Sixth Australasian Soilborne Diseases Symposium

Two features of modern science are its fragmentation into disciplines and a necessity to specialise. Thus even in areas as specific as soilborne diseases, scientists tend to see themselves as plant pathologists, mycologists, nematologists, microbiologists, soil ecologists or molecular biologists. Also, knowledge is often limited to a few pathogens on one or two crops.

Although some specialisation is necessary if we are to continue to improve our understanding of the complex belowground world, those working on soilborne diseases cannot afford to ignore the broader picture. We may be working on one specific aspect of a problem, but we need to recognise that numerous pathogens and a myriad of competitors interact within the root zone and these interactions are influenced by many factors, including moisture, temperature and the soil’s physical and chemical environment. We also need to recognise that our current cropping systems are the result of years of research and numerous inputs from growers, so new and potentially useful management practices must pass the test of being practical, profitable and sustainable.

The purpose of this meeting is to encourage interaction between scientists with disparate skills but a common interest in soil biology and soilborne diseases. The expertise of participants covers a wide range of fields, so please take the opportunity to discuss your work with as many of our delegates as possible. Hopefully you will leave with many new ideas and some collaborative arrangements that will add value to your research and extension programs.

Enjoy your three days at Twin Waters!

Graham Stirling
Chair, Organising Committee, 6ASDS

Organising committee
Dr Graham Stirling, Biological Crop Protection, Moggill
Ms Jenny Cobon, Agri-Science Queensland, Indooroopilly
Dr Olufemi Akinsanmi, University of Queensland, Indooroopilly
Dr Linda Smith, Agri-Science Queensland, Indooroopilly
Dr Rob Magarey, BSES Limited, Tully
Mr Jason Sheedy, Agri-Science Queensland, Toowoomba
Dr Marcelle Stirling, Biological Crop Protection, Moggill
Mr Wayne O’Neill, Agri-Science Queensland, Indooroopilly

Conference Organiser
Sally Brown Conference Connections
PO Box 108 Kenmore QLD 4069 Australia
Sally.brown@uq.net.au
# PROGRAM

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
<th>Page number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday 9 August</td>
<td>Registration and morning tea sponsored by CRDC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>Opening of symposium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1100</td>
<td>Biological interactions in soil</td>
<td>David Coleman</td>
<td>1</td>
</tr>
<tr>
<td>1200</td>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1300</td>
<td>New insights into the structure and function of microbial communities</td>
<td>James Tiedje</td>
<td>4</td>
</tr>
<tr>
<td>1400</td>
<td>New technologies to better understand ecological processes and community dynamics</td>
<td>Pauline Mele</td>
<td>15</td>
</tr>
<tr>
<td>1430</td>
<td>Afternoon tea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>Chair: Graham Stirling</td>
<td></td>
<td></td>
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<tr>
<td>1100</td>
<td>Understanding soil processes: one of the last frontiers in biological and ecological research</td>
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<tr>
<td>1200</td>
<td>Lunch</td>
<td></td>
<td></td>
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<tr>
<td>Session 2</td>
<td>Chair: Kathy Ophel-Keller</td>
<td></td>
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</tr>
<tr>
<td>1300</td>
<td>Restoration of organic carbon in soil</td>
<td>Ralph Noble</td>
<td>2</td>
</tr>
<tr>
<td>1400</td>
<td>Risks and benefits of using compost as organic soil amendments</td>
<td>Peter McGee</td>
<td>10</td>
</tr>
<tr>
<td>1430</td>
<td>Afternoon tea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 3</td>
<td>Poster session</td>
<td>Welcome reception and dinner</td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td>Postsession</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuesday 10 August</td>
<td>Chair: Matthew Cromey</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0900</td>
<td>Restoration of organic carbon in soil</td>
<td></td>
<td></td>
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<tr>
<td>0930</td>
<td>Risks and benefits of using compost as organic soil amendments</td>
<td>Ralph Noble</td>
<td>2</td>
</tr>
<tr>
<td>0950</td>
<td>Microbial sequestration of organic carbon</td>
<td>Peter McGee</td>
<td>10</td>
</tr>
<tr>
<td>1010</td>
<td>Potential for biochar in soilborne disease management</td>
<td>Lukas Van Zweiten</td>
<td>25</td>
</tr>
<tr>
<td>1030</td>
<td>Morning tea sponsored by SRDC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 4</td>
<td>Chair: Olufemi Akinsanmi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1100</td>
<td>Characterisation of <em>Rhizoctonia solani</em> anastomosis group 2-1 from potato tubers in New Zealand</td>
<td>Subha Das</td>
<td>41</td>
</tr>
<tr>
<td>1115</td>
<td>Genetic diversity of <em>Plasmodiophora brassicae</em> in Australia</td>
<td>Abdelwahab Badi</td>
<td>32</td>
</tr>
<tr>
<td>1130</td>
<td>Progress in comparing <em>Fusarium pseudograminearum</em> infection levels and crown rot symptoms in stem internodes of cereals</td>
<td>Jill Petrisiko</td>
<td>74</td>
</tr>
<tr>
<td>1145</td>
<td>Does addition of the element silicon affect the infection process of <em>Fusarium oxysporum</em> f. sp. <em>cubense</em> on banana?</td>
<td>Kevan Jones</td>
<td>58</td>
</tr>
<tr>
<td>1200</td>
<td>Response of soil microfloral communities to stubble addition differs between suppressive and non-suppressive soils</td>
<td>Vadakattu Gupta</td>
<td>50</td>
</tr>
<tr>
<td>1215</td>
<td>Identifying QTL for Fusarium crown rot resistance (<em>F. graminearum</em>) in two spring wheat populations (Sunco/Macon and Sunco/Otis)</td>
<td>Grant Poole</td>
<td>76</td>
</tr>
<tr>
<td>1230</td>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session</td>
<td>Date</td>
<td>Time</td>
<td>Chair</td>
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<tr>
<td>Session 6</td>
<td>1330-1430</td>
<td>1300-1400</td>
<td>Russell Eastwood, Hugh Wallwork, Richard Trethowan</td>
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<tr>
<td></td>
<td></td>
<td>1430-1500</td>
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<td></td>
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<tr>
<td>Session 7</td>
<td>1530-1630</td>
<td>1530-1630</td>
<td>Jason Sheedy</td>
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<td>1630-1700</td>
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<td>1830-1900</td>
<td></td>
</tr>
<tr>
<td>Session 8</td>
<td>0900-1000</td>
<td>0900-0915</td>
<td>Alison Stewart</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0915-1000</td>
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<tr>
<td>Session 9</td>
<td>1330-1430</td>
<td>1330-1400</td>
<td>Richard Falloon</td>
</tr>
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<td>1630-1700</td>
<td></td>
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<tr>
<td>Session 10</td>
<td>1330-1430</td>
<td>1330-1400</td>
<td>Kirsty Owen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1400-1430</td>
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The future of the Australasian Soilborne Diseases Symposium (ASDS)

The first ASDS was held in February 1999 on the Gold Coast (Queensland) and was followed by meetings in Lorne (Victoria), the Barossa Valley (South Australia), Queenstown (New Zealand) and Thredbo (New South Wales). These symposia have proved to be a valuable forum for those interested in soil health, soilborne diseases and soil biology. Our delegates have come from many countries and have worked on a wide range of crops, pathogens and beneficial organisms in diverse environments. Some have been experts in specific areas such as soil ecology and molecular biology while others have had a broader role in agronomy, extension, teaching or the commercial aspects of agriculture. In an era where science is becoming increasingly specialised, this interaction between people with different skills and backgrounds has contributed to the success of ASDS.

Previous arrangements for organizing ASDS have been quite informal, and we would like to see it remain that way. Although we don’t have a constitution or a management committee, the current system has worked well, as we have always been able to find a group of people prepared to organise the next symposium. This has allowed flexibility in the choice of meeting location, content of the scientific program and meeting theme, and ensured that ASDS has continued to evolve and remain relevant.

Since the meeting in Thredbo 18 months ago, we have made the following arrangements in the hope that they will make it easier to organise future symposia:

- ASDS is now a formal sub-group within the Australasian Plant Pathology Society (APPS). This ensures that ASDS has access to the APPS website and can readily communicate with the wider plant pathology community, both in Australasia and elsewhere.
- Arrangements have been made to deposit any profits from symposia in an APPS account. Not only will this provide accountability, but it will ensure that start-up funds are available to the organising committee of the next ASDS.
- ASDS keynote papers have always been published in Australasian Plant Pathology, but the issue of whether ASDS is liable for page charges has never been fully clarified. Arrangements have now been made to ensure that our keynote papers are treated in the same way as keynote papers at APPS conferences.
- Within three months of the completion of 6ASDS, the current organising committee will report on the meeting, provide a profit/loss statement and summarise the results of a questionnaire completed by delegates. We hope that future committees will see such reporting as a worthwhile endeavour, as it should assist those planning the next symposium.

We hope that these arrangements will help ensure that ASDS has a long term future and that it remains relevant to all those interested in soilborne pathogens and the complex world they inhabit.

Graham Stirling and Rob Magarey
Australasian Soilborne Diseases Symposium
Keynote papers

Abstracts of keynote papers are presented in these Proceedings.
Complete versions of these papers will be published in Australasian Plant Pathology
(volume 40, first issue of 2011)

Biographical sketches of keynote speakers

David Coleman is Distinguished Research Professor Emeritus, University of Georgia, Athens, Georgia, USA. David obtained his PhD from the University of Oregon and after five years at the University of Georgia, moved to the University of Colorado at Fort Collins. During his time in Colorado (1972-1985) David played a key role in perhaps the world’s most influential soil ecology group, contributing to our understanding of biotic interactions in the rhizosphere; food web structure and function; organic matter decomposition and turnover; and nutrient dynamics in soil. On his return to the University of Georgia in 1985, he continued his research on energetics, decomposition, nutrient cycling and soil biodiversity. In 1996 he co-authored Fundamentals of Soil Ecology, which became an important reference text for scientists and students with an interest in soil biology and ecology. In 1979-80 he was Senior Research Fellow with the Soil Bureau at Lower Hutt, New Zealand, and in 2006, a McMaster Visiting Fellow with CSIRO in Adelaide, South Australia.

James Tiedje is Distinguished Professor of Microbiology and Molecular Genetics Michigan State University, East Lansing, Michigan, USA. After degrees from Iowa State University and Cornell University, James’ research has focused on microbial ecology, physiology and diversity, especially with regard to the nitrogen cycle, biodegradation of environmental pollutants and the use of molecular and genomic approaches to understand microbial community function. He was Editor of Applied and Environmental Microbiology and Microbial and Molecular Biology Reviews, served on the National Research Council’s Board on Life Sciences and Co-Chaired a Committee on the new science of Metagenomics. James was President of the American Society for Microbiology and the International Society of Microbial Ecology and is a member of the U.S. National Academy of Sciences. He shared the 1992 Finley Prize from UNESCO for research contributions in microbiology of international significance and was recently awarded an Einstein Professorship by the Chinese Academy of Sciences.

Ralph Noble is Professor and Principal Investigator at Warwick HRI (formerly Horticultural Research International but now part of the University of Warwick) in Wellesbourne, UK. He obtained his BSc from the University of Reading and a PhD from Cranfield University. After a short period of postdoctoral research at the Institut für Landtechnik at Bonn University, Germany, Ralph has worked in applied crop research in the UK since 1984. He was previously based at HRI Littelehampton and has been at Wellesbourne since 1994. His main research interests are: suppressing plant pathogens using composts; control of soil-borne plant pathogens using biocontrol agents; examining the survival of plant pathogens during composting; recycling wastes to produce pest-free horticultural growing media; reducing composting odours; and mushroom cultivation. He has been involved in horticultural research and development projects in several countries in Europe, as well as the USA, Mauritius, China, New Zealand and Australia.
# TABLE OF CONTENTS

## Keynote Speakers

**Understanding Soil Processes: One of the Last Frontiers in Biological and Ecological Research**  
*Coleman D C*  
1

**Risks and Benefits of Using Composts as Organic Soil Amendments**  
*Noble R*  
2

**New Technologies to Better Understand Ecological Processes and Community Dynamics**  
*Tiedje J M*  
4

## Invited Speakers

**Sustainable Farming Systems – Key Management Factors and Their Application in Subtropical and Tropical Vegetable Production Systems**  
*Bell M J, Pattison, A B and Harper S*  
5

**Options for Enhancing Resistance to Soilborne Diseases of Cereals: A Commercial Plant Breeder’s Perspective**  
*Eastwood R*  
8

**Microbial Sequestration of Organic Carbon**  
*McGee P A*  
10

**Use of Molecular Diagnostics for Improved Decision-Making by Growers**  
*McKay A C and Ophel Keller K M*  
11

**Harnessing the Biological Potential of Australia’s Grain Growing Soils**  
*Mele P M and Blumenthal M J*  
13

**Potential Applications of Soil Microbial Metagenomics**  
*Mele P M*  
15

**International Research and Capacity Building for the Control of Soilborne Pathogens in Rain-Fed Wheat Production Systems.**  
*Nicol J M, Bolat N and Braun H J*  
17

**New Approaches to Detecting Phytophthora**  
*O’Brien PA*  
19

**Importance of Soil Organic Matter to Soil Health and Disease Suppression in Vegetable Crops**  
*Porter I J, Mattner S W and Edwards J*  
20

**Understanding Variability in Biocontrol Systems**  
*Stewart A*  
22

**An International Perspective on Breeding for Resistance to Soil Borne Pathogens**  
*Trethowan R, Mathews K, Manes Y and Nicol J M*  
24

**Potential for Biochar in Soilborne Disease Management**  
*Van Zwieten L*  
25

**Strategies for Enhancing Resistance to Soilborne Pathogens of Cereals: A Plant Pathologist’s Perspective**  
*Wallwork H*  
27
ORAL AND POSTER PRESENTATIONS

STRESS PREDISEPOSES MACADAMIA ROOTS TO PHYTOPHTHORA INFECTION
  O A Akinsanmi and A Drenth

USE OF TELONE C35 TO REDUCE SOILBORNE RHIZOCTONIA INOCULUM FOR MANAGEMENT OF ONION STUNT
  S T Anstis, S J Pederick and T J Wicks

A DESCRIPTIVE MODEL FOR IMPROVED MANAGEMENT OF CROWN ROT OF WHEAT
  D Backhouse

A MECHANISTIC MODEL FOR THE SPREAD OF CROWN ROT IN CONTINUOUS WHEAT
  D Backhouse

GENETIC DIVERSITY OF PLASMODIOPHORA BRASSICAE IN AUSTRALIA
  A Badi, A C Lawrie and EC Donald

EFFECT OF THE BIOPESTICIDE BACILLUS THURIENGENSIS ON POPULATIONS OF NON-TARGET NEMATODES
  N L Bell and L T Aalders

ISOLATION AND CHARACTERISATION OF POTENTIAL BACTERIAL BIOCONTROL AGENTS FROM BRASSICA AND POTATO CROPPING SYSTEMS
  M Braithwaite, E Hicks, A Stewart, L Loguercio, R E Falloon and D Bienkowski

BIOLOGICAL CONTROL OF RHIZOCTONIA SOLANI IN PERENNIAL RYEGRASS USING TRICHODERMA ATROVIRIDE ISOLATES
  P K Chohan, D R W Kandula, A Stewart and J G Hampton

SOIL AND SEED Mn EFFECTS ON TAKE-ALL
  S L Bithell, D Curtin, A McKay, M G Cromey

SOIL pH AND Ggt INOCULUM LEVEL EFFECTS ON TAKE-ALL
  S L Bithell, D Curtin, R C Butler, A McKay and M G Cromey

SEED POTATO CERTIFICATION: ITS VALUE TO INDUSTRY
  N S Crump and D ftMarshall

EVALUATION OF THE EFFICACY OF AVICTA AS SEED TREATMENT ALONE OR IN COMBINATION WITH FUSARIUM OXYSPORUM STRAIN 162 FOR MANAGEMENT OF ROOT-KNOT NEMATODE ON TOMATO
  A A Dababat, C Watrin, A Cochran, M Klix and R A Sikora

SOILBORNE DISEASES IMPACTING AVOCADO PRODUCTION IN AUSTRALIA
  E K Dann, L A Smith and K G Pegg

CHARACTERISATION OF RHIZOCTONIA SOLANI ANASTOMOSIS GROUP 2-1 FROM POTATO TUBERS IN NEW ZEALAND
  S Das, F A Shah, R E Falloon, R C Butler and A R Pitman

RESISTANT VARIETIES AS A MANAGEMENT TOOL FOR THE POTATO CYST NEMATODE (GLOBODERA ROSTOCHIENSIS) IN VICTORIA, AUSTRALIA.
  R F de Boer, N S Crump, F Thomson, W S Washington, D V Beardsell and A L Yen

THE POTENTIAL OF BIOFUMIGANT AND GREEN MANURE CROPS AS A TOOL TO MANAGE SOILBORNE DISEASES IN VEGETABLE PRODUCTION
  E C Donald, O N Villalta, C A Scoble, D Wite, D Riches, S Mattner, V Chandolu, R B Jones, M Imsic, D Allen and I J Porter
WHEAT GENETIC RESISTANCE TO DRYLAND CROWN ROT (Fusarium culmorum) FROM INVITRO SEEDLING AND ADULT PLANT SCREENING

G Erginbas, J M Nicol and E Kinaci

VISUAL DISEASE ASSESSMENT AS A RESEARCH TOOL – A CASE STUDY

M L Evans and H Wallwork

ELEVATED ZINC AND MANGANESE LEVELS GIVE MODERATE REDUCTIONS IN SPONGOSPORIA SUBTERRANEAE INFECTION OF POTATO ROOTS

R E Falloon, D Curtin, R A Lister, R C Butler, C L Scott and N S Crump

ARE ORGANIC FARMING SOILS MORE DISEASE SUPPRESSIVE?

P F Geense, L M Forsyth, T Kukulies, A B Pattison and A B Molina

POPULATION GENETICS OF THE PLANT PATHOGENIC PROTOZOAN SPONGOSPORIA SUBTERRANEAE F.SP. SUBTERRANEAE

R D Gau, B A McDonald, U Merz, P C Brunner and R E Falloon

SUPPRESSION OF DAMPING-OFF OF RADISH CAUSED BY RHIZOCTONIA SOLANI AG2.1 WITH SOIL CARBON AMENDMENTS


RESPONSE OF SOIL MICROFLORAL COMMUNITIES TO STUBBLE ADDITION DIFFERS BETWEEN DISEASE SUPPRESSIVE AND NON-SUPPRESSIVE SOILS

V V S R Gupta and N P E Reddy

TEMPORAL DYNAMICS OF RHIZOCTONIA SOLANI AG8 INOCULUM IN AUSTRALIAN SOILS

V V S R Gupta, A McKay, S Diallo, D Smith, A Cook, J Kirkegaard, K Ophel-Keller and D K Roget

BACTERIAL INOCULATION OF BANANA IMPROVES PLANT GROWTH UNDER REDUCED FERTILISER TREATMENT

S D Hamill and E Rames

SPATIAL DISTRIBUTION OF THE SOIL BORNE PATHOGEN COLLETOTRICHUM COCCODES AND SUBSEQUENT DISEASE EXPRESSION ON POTATOES AT HARVEST

R Harding, A Benger, C Todd, Herdina, A Mckay and K Ophel-Keller

A BIOASSAY TO SCREEN BIOLOGICAL CONTROL AGENTS AGAINST AERIAL INFECTIONS OF SCLEROTINIA SCLEROTIORUM ON BRASSICA LEAVES

E Hicks, M Braithwaite, M Pan and A Stewart

FIELD CROP NEMATOLOGY IN SOUTH-EASTERN AUSTRALIA

G J Hollaway, A B Purdue and A C McKay

YIELD LOSS CAUSED BY CROWN ROT IN CEREALS IS RELATED TO PRE-SOWING SOILBORNE PATHOGEN LEVELS AND RAINFALL

G J Hollaway, G K Exell and A C McKay

RHIZOSPHERE BACTERIA ASSOCIATED WITH TWO GRAPEVINE ROOTSTOCKS THAT VARY IN SUSCEPTIBILITY TO CYLINDROCARPON BLACK FOOT DISEASE

D S Dore, E E Jones, H J Ridgway and M V Jaspers

DOES ADDITION OF THE ELEMENT SILICON AFFECT THE INFECTION PROCESS OF Fusarium oxysporum F. Sp. Cubense ON BANANA?

K W Jones, B Cribb and E A B Aitken
EFFECT OF TREHALOSE ON THE BIOLOGICAL ACTIVITY OF TRICHODERMA 
ATROVIRIDE, LU132  
J Kandula, M Braithwaite, A Hay and A Stewart  
59

GROWTH PROMOTION AND BIOLOGICAL CONTROL OF RHIZOCTONIA SOLANI 
IN OILSEED RAPE USING BENEFICIAL BACTERIAL ISOLATES  
D R W Kandula, A Stewart, M Braithwaite and J G Hampton  
60

HISTOPATHOLOGICAL INVESTIGATION OF FUSARIUM CROWN ROT IN WHEAT  
N L Knight, A Lehmensiek, D J Herde and M W Sutherland  
61

PYTHIUM SPP. ON GINGER (ZINGIBER OFFICINALE ROSCOE) IN AUSTRALIA  
P D Le, M K Smith and E A B Aitken  
62

A COMPARISON OF NEMATODE COMMUNITIES IN VERTOSOLS UNDER CROP 
AND PASTURE FROM THE DARLING DOWNS, QUEENSLAND  
Y Li and G R Stirling  
63

BIOLOGICAL FACTORS INFLUENCE NEMATODE DISTRIBUTION IN 
VERTOSOLS FROM THE NORTHERN GRAIN-GROWING REGION  
Y Li and G R Stirling  
64

MONITORING ROOT AND LEAF SALICYLIC ACID TO OPTIMISE INDUCTION OF 
SYSTEMIC ACQUIRED RESISTANCE IN BROCCOLI  
D Lovelock, A Agarwal, E C Donald, I J Porter and D M Cahill  
65

PROPAMOCARB: MANAGING DAMPING-OFF IN PAPAYA  
M F Male and L L Vawdrey  
66

SOIL UTILITY OF A UNIQUE STRAIN OF BACILLUS SUBTILIS, QST 713, FOR 
DISEASE CONTROL, CROP YIELD AND QUALITY IMPROVEMENTS  
D C Manker, E Martinez, D Long, D Warkentin, P Walgenbach, D Silva, M Guilhabert 
and S Lego  
67

THE POTENTIAL OF SESAME OIL EXTRACTS FOR MELOIDOGYNE JAVANICA 
CONTROL  
J W McCarthy, E A B Aitken, M J Furlong and J A Cobon  
68

ADAPTED SPRING AND WINTER WHEATS WITH RESISTANCE AGAINST 
MULTIPLE SOILBORNE PATHOGENS (CEREAL NEMATODES – HETERODERA FILIPJEVI 
AND PRATYLENCHUS SPP. AND CROWN ROT - FUSARIUM CULMORUM) TARGETED 
FOR RAINFEED WHEAT PRODUCTION SYSTEMS  
J M Nicol, N Bolat, G Erginbas, A A Dababat, A Yorganicilar, İ H Elekcioglu, 
E Sahin and H Toktay  
69

EFFICACY OF LOQUAT SEED TO CONTROL ROOT-KNOT NEMATODES IN 
VEGETABLES  
W T O’Neill, J A Cobon, A B Pattison and G W Berry  
70

CHANGES IN POPULATION DENSITIES OF MERLINIUS BREVIDENS IN A 
4-YEAR SUMMER CROP ROTATION EXPERIMENT  
K J Owen, T G Clewett and J P Thompson  
71

CROWN ROT RESISTANCE IN BREAD WHEAT SIGNIFICANTLY ELEVATED 
THROUGH GENETIC RESEARCH  
D J Herde, C D Percy and T L Walters  
72

 FUNGI AND OOMYCETES ASSOCIATED WITH ROOT ROT COMPLEX IN 
PARSNIP CROPS  
J E Petkowski, E J Minchinton, R F de Boer and F Thomson  
73
THE IMPACT OF CROP ROTATION, TILLAGE PRACTICE AND ORGANIC AMENDMENTS ON SUPPRESSIVENESS TO ROOT-KNOT NEMATODE AND FUSARIUM WILT

G R Stirling, M K Smith and A M Stirling

STUDIES ON THE EFFECTIVENESS OF TRICHODERMA AND SOIL AMENDMENTS AGAINST STEM AND POD ROT CAUSED BY SCLEROTIUM ROLFSII IN GROUNDNUT

G Sunkad

THE ROLE OF PEROXIDASE IN BASAL RESISTANCE OF SUGAR BEET AGAINST THE RHIZOCTONIA ROOT ROT DISEASE

P Taheri and S Tarighi

MOLECULAR AND CYTOLOGICAL ASPECTS OF TOMATO-RHIZOCTONIA SOLANI INTERACTION

P Taheri and S Tarighi

INVOLVEMENT OF PHENYLPROPANOID SIGNALING IN DEFENSE RESPONSES OF SUGAR BEET TO A NECROTROPH PATHOGEN

P Taheri and S Tarighi

HYDROPONICS ENABLES PRECISE IDENTIFICATION OF INFECTION WINDOW IN COMMON SCAB DISEASE OF POTATO

B B Khatri, R S Tegg, P H Brown and C R Wilson

2,4-DICHLOROPHENOXYACETIC ACID INDUCED RESISTANCE TO COMMON SCAB OF POTATO

H K Thompson, R S Tegg and C R Wilson

POLYMYXA GRAMINIS IN A CEREAL CROP IN AUSTRALIA

J P Thompson, T G Clewett, R Jennings, J G Sheedy, K J Owen and D M Persley

GENETIC RESISTANCE IN WHEAT TO ROOT-LESION NEMATODE (PRATYLENCHUS THORNEI)

J P Thompson, T G Clewett and J G Sheedy

QUANTIFYING TUBER- AND SOIL-BORNE INOCULUM OF RHIZOCTONIA SOLANI IN POTATO PRODUCTION SYSTEMS IN NEW ZEALAND

S E Thompson, S Keenan, T Nelson, P J Wright and A R Pitman

THE ROLE OF ROTATION CROPS IN MANAGING PLANT-PARASITIC NEMATODES ON GINGER IN FIJI

U Turaganivalu, G R Stirling and M K Smith

ROOT ROT OF GREEN BEANS CAUSED BY APHANOMYCES EUTEICHES: SYMPTOMS, DETECTION AND MANAGEMENT IMPLICATIONS

A Watson, L Browne and M Snudden

INVESTIGATING THE ROLES OF RHIZOCTONIA SOLANI AG2.1 AND 3 IN CAUSING STOLON PRUNING AND STEM CANKER IN POTATOES

T J Wiechel, M Wardzynski, J Verstraten and F Richardson

COLLECTION AND IDENTIFICATION OF TRICHODERMA SPECIES IN GREEN SPACES OF TEHRAN

J Zad, M Kiarudi and D Zafari

INTERACTION OF VERTICILLIUM DAHLIAE AND MELOIDOGYNE JAVANICA IN SENSITIVE AND RESISTANT OLIVE SEEDLINGS

I Zad, A Saedizadeh and A Kheiri
Soils are one of the great unknown realms on earth, despite decades of extensive research. We still see soils ‘through a ped darkly’ (Coleman 1985). This opacity in milieu and understanding rewards innovative study, however, as soils are ‘complex adaptive systems’ (Young and Crawford 2004; Crawford et al. 2005), with sophisticated levels of self-organization.

Viewed historically, soil ecological studies have progressed from what major groups of biota are present, what is their biomass, and what major processes occur. More recent studies have delineated multi-trophic interactions, extending both above- and below-ground, as well as specifically-targeted studies of substrates and organisms that are involved in the development and function of suppressive soils. One of the great unknowns in soil ecology is a fuller understanding of the complete array of predatory biota. Soils are teeming with organisms in all three Domains, but are also rife with many phages and other viruses infecting Archaea and Eukarya. Pursuing a more holistic approach including viral biology and ecology may enable us to more capably manage our soils that have supported the biosphere so much over the millennia.

Looking into the future, the opportunity to exploit soil biodiversity in the context of ecosystem development should pay considerable dividends. Following the fungal: bacterial ratios in ecosystem successions, sensu Harris (2009) deserves further exploration. Metatranscriptomics, i.e., the measurement of genomes that are active at any point in time, should be explored by soil ecologists. Using chronosequence analysis, the relationships between soil biodiversity and ecosystem function are beginning to be understood. Finally, management of the plant-soil-microbial-faunal food web via various organic amendments shows possibilities in the study and management of suppressive soils. I look forward to stimulating presentations on these topics during the meeting.

References
RISKS AND BENEFITS OF USING COMPOSTS AS ORGANIC SOIL AMENDMENTS

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The supply of composts has increased in many countries due to the enforced diversion from landfill of organic biodegradable wastes. These include green wastes such as yard trimmings and reject fruit and vegetables, carbon-rich materials such as paper and wood wastes, nitrogenous wastes such as animal manures and sewage sludge, and increasing quantities of food wastes. Often the primary financial incentive for composting is for this organic waste disposal, with income from low value end-products such as organic soil amendments being a secondary or negligible consideration. Composts can have a significant agricultural benefit, particularly on impoverished soils, in regions with limited rainfall, and in organic agriculture, where the use of synthetic fertilisers is not permitted. These benefits include the supply of plant nutrients, particularly P and K, increased soil organic matter, moisture retention, and cation exchange capacity, improved soil structure, and suppression of soil-borne diseases and weeds. However, the bulkiness of compost means that transport for use as an organic soil amendment is usually only viable over short distances. Regulations such as the EU nitrates directive (Anon, 1991) can limit permitted compost application rates to below those which result in significant benefit, at least in the short term. The use of composts can also pose risks such as those caused by contents of toxic elements and compounds such as herbicide residues, populations of plant and animal pathogens which may have survived the composting process, and man-made inert such as glass and plastic (Noble et al., 2009). Composts may contain high levels of soluble salts or be too alkaline or immature, leading to the immobilisation of soil nitrogen and/or phytotoxicity caused by organic acids and other volatile organic compounds. Compost variability can also be a significant problem, leading to unpredictable crop response. This variability can be measurable such as nutrient content and salinity, but the causes and measurement of compost variability in relation to factors such as nitrogen supply and immobilisation, and disease suppressiveness may be elusive (Noble and Coventry, 2005). Greater control over variables in the composting process, such as in the selection and rejection of feedstock wastes, moisture content adjustment and in allowing for an adequate maturation period, can improve compost quality and uniformity although cost implications must also be considered if compost use as a soil organic amendment is to be viable. The introduction of compost quality standards such as PAS 100 in the UK (Anon., 2005) has been aimed at reducing risks to the compost end user and improving confidence in compost use. Composts can be incorporated into the soil profile or used as a surface mulch. Often the best methods and timing of compost application in the field have yet to be established for particular crops, cropping rotations, soil types, and locations.

Research at Warwick HRI has focused on reducing the risks posed by the plant pathogen content of composts, and improving the understanding, efficacy and reliability of disease suppression resulting from soil amendment with composts. The temperature and exposure time in compost required for eradicating a range of plant pathogens with hardy resting spores such as Plasmodiophora brassicae and Fusarium oxysporum f. sp. brassicae, or sclerotia such as Sclerotinia sclerotiorum and Sclerotium cepivorum has been established in both controlled laboratory and large-scale composting tests. The development of indicator organisms which can be inserted in compost and tested for subsequent viability has been used to augment time-temperature data for testing the sanitisation of composting wastes.

The suppression of soil-borne pathogens has frequently been shown to be due to microbial antagonism by demonstration of loss of suppressiveness following compost sterilisation (Noble and Coventry, 2005). However, abiotic factors such as increases in soil pH following compost amendment have also been correlated with control of wilt diseases.
caused by *F. oxysporum* and clubroot of *Brassicas* caused by *P. brassicae* (Termorshuizen *et al.*, 2007; Noble *et al.*, 2006). Composted onion waste, a significant disposal problem for the onion industry, has been shown to retain the onion volatiles that stimulate the germination and subsequent death of resting sclerotia of the Allium white rot pathogen, *S. cepivorum*, before an onion crop is planted in infested soil. A significant problem in the biological control of soil-pathogens in the field has been the achievement of sufficiently high soil populations of biocontrol agents at an economically viable cost. Composts that support the growth of biocontrol agents such as *Trichoderma viride* have been used to increase the soil population of these beneficial microorganisms to levels which give reproducible levels of control of both Allium white rot (Coventry *et al.*, 2006) and Fusarium basal rot caused by *F. oxysporum f.sp. cepae*.

**References**


NEW TECHNOLOGIES TO BETTER UNDERSTAND ECOLOGICAL PROCESSES AND COMMUNITY DYNAMICS

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Advances in ‘Omics’ technologies is giving unprecedented insight into the biological world, and has been particularly impactful for microbial ecology since for the first time we can have a more comprehensive view of community structure, gene composition and at least some information on activity through expressed proteins and metabolic fluxes. These advances have been driven by advances in sequencing technologies, which have increased the capacity and lowered the cost, i.e. the democratisation of sequencing. A decade ago we were analysing Kb of sequence, a few years ago Mb, now Gb, and next year perhaps Tb. The problem has become how to analyse such massive data sets, not its generation. It is also changing the expertise needed for microbial ecology to one in which coding, computation, high throughput pipelines, and visualization tools are the daily activity. Nonetheless, the biological insight and questions must remain front and centre so that the most important knowledge is gained from the new technologies.

In microbial ecology we can now use these technologies to do certain things well. We can more comprehensively determine community structure to much greater depth and replication, and use that information to assess community differences over time and space, and correlate those differences with environmental attributes. We can also learn about the types and amounts of genes associated with key functions in communities by amplicon (gene-targeted) pyrosequencing (Iwai et. al., 2009) or microarray (GeoChip) technologies (He et. al., 2007). These are particularly useful for genes directly involved in biogeochemical cycles, cell signalling, pathogenicity, antibiotic resistance and biodegradation, for example, and will at some future period allow sequences to be diagnostic markers for ecologically important functions. Shotgun metagenomics, first used in marine microbial ecology, but now beginning to be used productively in soil (the most complex habitat) provides the catalogue for all genes in a community, some of which will reflect the selection that led to their occurrence. The current challenge for using metagenomics in soil is that its complexity makes it difficult to obtain sufficient assembly to interpret function. Deeper sequencing with the more advanced sequencing methods is beginning to make some progress on this key front. The deeper sequencing is also providing improved insight into expression using RNASeq, an approach we used with a bacterial culture under soil-inducing conditions (Yoder-Himes).

I will illustrate the uses and understanding gained from these methods in several studies including the rhizospheres of different crops, the effect of land-use change, and the effect of different ecosystems on the composition of targeted functional genes and taxa.

References
INVITED SPEAKERS

SUSTAINABLE FARMING SYSTEMS – KEY MANAGEMENT FACTORS AND THEIR APPLICATION IN SUBTROPICAL AND TROPICAL VEGETABLE PRODUCTION SYSTEMS

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Principles developed in broadacre cropping

The issue of farming system sustainability is essential to the long term food security of Australia and our trading partners. Research in the broadacre grains and sugarcane industries (Bell et al. 2007; Stirling 2008) has highlighted some key characteristics of land management that contribute to such sustainability, and developed frameworks against which management practices should be evaluated. Key aspects include maintenance or enhancement of soil organic matter, minimizing or controlling soil compaction, reduction or elimination of aggressive tillage, maintaining chemical fertility by balancing nutrient removals with nutrient inputs and adopting a crop rotation strategy.

Foremost among these is the conservation or enhancement of soil organic matter status. Demonstrated benefits include enhanced general suppression of plant-parasitic nematodes and other soilborne pathogens, better soil structure and greater storage and in-crop mineralisation of nutrients like nitrogen. A key issue for capturing the benefits of organic matter inputs appears to be the continuity of organic matter addition and its subsequent decomposition (both to soil surface and deeper in the soil profile), with annual surface crop residue inputs only providing benefits in the shallow soil surface layers.

Adoption of reduced or zero tillage provides significant advantages, but adoption is restricted unless compaction can be eliminated or controlled. Therefore development of agronomic practices that allow system-wide traffic compatibility are essential precursors before the full benefits of reduced or zero tillage can be captured. While tillage continues, organic matter decomposition is accelerated so higher addition rates are needed to maintain the microbial community, and the constituents of the microbial community are altered. Key features of that modification are an accelerated rate of mineralisation (due to the predominance of bacteria over fungi) and a loss of suppression associated with the fungal component of the microbial community.

Chemical fertility maintenance is challenged in all broadacre systems. In the grains industries the key issue is driven by low crop gross margins and large seasonal production risks, resulting in generally negative nutrient budgets and declining native fertility reserves. By contrast, sugar farming systems are challenged by quite large but often unbalanced nutrient inputs such that soil nutrient reserves may be increasing or decreasing, dependent on the nutrient involved. While both industries are reasonably well served with diagnostic criteria to identify fertilizer requirements, there are challenges in determining appropriate application rates. There are also emerging problems with quantification of the slowly available nutrient reserves that have been buffering soils against negative crop nutrient budgets.

Finally, availability and deployment of suitable rotation species in both cropping systems is demonstrably suboptimal. The grains industry in the subtropics is challenged by the lack of profitable and adapted non-cereal crop options (especially grain legumes), while the sugar industry has been generally slow to recognise the value of rotation breaks and hence develop suitable rotation strategies. The result is that both industries experience significant productivity losses and reduced input use efficiencies due to pathogen activity and poor root system health.
Slow adoption rates of sustainable farming practices in intensive vegetable systems

Although vegetables are usually treated generically as a commodity, there are at least 20 major vegetable crops and often very little commonality with regard to how they are produced. Vegetable production systems also have a high cost structure, with a large array of inputs (e.g. machinery, transport, refrigeration, packaging, nutrients, pesticides, plastic mulch and drip irrigation) used to maximise crop productivity and profitability, at least in the short term. Furthermore, modern vegetable marketing arrangements require producers to deliver large quantities of high-quality, consistent product on a daily basis whilst ensuring that stringent food safety standards are achieved. Vegetable production systems are therefore intensive to the extreme. In Queensland, year round production of most major commodities is achieved across the complement of its tropical and subtropical climatic zones.

From a soils perspective, several key factors impact on soil management practices in tropical and subtropical vegetable production. Tight rotations and intensive cultivation are a feature of most vegetable farming systems, but because temperatures are relatively high throughout the year, soil organic carbon is rapidly degraded, leading to associated soil biology and fertility problems. High pesticide and nutrient inputs are used to overcome these problems, but in an environment where extreme rainfall events are a regular occurrence, there is always a danger of off-site movement.

In comparison with field crop industries, the vegetable industry appears to have been slow to adopt improved and sustainable soil management practices. However, the potential to implement such practices is confounded by a range of complexities that greatly impact on the profitability of vegetable production. These include the large array of crop types and their associated agronomies, variation in soil types, the immediacy in timing of farming operations, the high perishability of the product and the intensity of inputs required to produce a high quality, blemish-free product. On a practical and logistical basis, these production conditions and constraints critically impact on the capacity of vegetable growers to change the way they manage their soils, setting the industry apart from other lower intensity agricultural production systems. Overall end-use market requirements determine the productivity and economics of vegetable farming and they are the single most important driver for growers attempting to improve their soil management practices.

Despite the challenges involved, good examples of improved soil management systems are evident in the vegetable industry, including waste amendment application, minimum tillage, controlled traffic and cover cropping. However, the implementation of these practices has tended to be crop specific. Thus minimum tillage has been successfully adopted in some vegetable rotations, particularly sweet corn and green beans, whereas the state of soil tilth required at planting and the trafficking that occurs over cultivated areas during harvest has meant that it has not been possible to modify tillage practices in crops such as lettuce. The commercialization of very accurate GPS in recent years is now offering the opportunity to establish permanent beds with reduced cultivation in vegetable crops where harvest aids are used.

Although nutrients are used excessively in some sectors of the vegetable industry, there are examples where improvements are being made. In vegetable production systems of the Lockyer Valley, for example, application of N is now largely fine-tuned to meet crop demand (Harper and Menzies 2010). However, under-application of potassium is a serious concern, as vegetable growers are drawing down natural soil reserves.

Indicators of more sustainable soil management practices

Over time, the farming practices used for traditional vegetable production have produced significant changes in soil physical, chemical and biological properties. Furthermore, they have altered the soil ecosystem by reducing soil biodiversity and the activity of soil organisms, with these changes impacting on suppressiveness to soil borne diseases. Studies of the changes in soil properties under contrasting managements provide an indication of the extent to which the soil ecology and resulting pathogen dynamics can be changed by
introducing new soil management practices into intensive vegetable systems. Such a scoping study was recently conducted across vegetable production areas in Queensland, New South Wales and Western Australia. ‘Conventional’ vegetable farming practices were compared with ‘alternate’ practices, which included various combinations of minimum tillage, mulching, organic amendments and controlled traffic, and significant changes in soil properties were observed. However changes were not always consistent across the various sites, with differences in soil type seeming to have a major influence on the impact of any particular management change (Pattison 2009).

Soil texture is a primary determinant of soil properties and is therefore an important factor in determining the impact of practices that might contribute to a sustainable production system. Sandy soils were found to be more amenable to change than heavy textured soils, as they tended to have lower organic C and lower biological diversity. Provided organic matter additions were repeated over a long period or large applications were made infrequently, organic amendments altered the properties of sandy soils, resulting in higher levels of organic carbon, greater microbial biomass C and an increase in the relative activity of soil bacteria.

The soils included in the study varied greatly in their nitrate and phosphorus contents, highlighting differences in the ability of producers to successfully balance nutrient inputs. High soil nutrient contents alter biological activity, change the composition of the microbial community and may impact on the soils ability to suppress soil borne diseases. Minimum tillage systems tended to have greater organic C than conventional soils, with greater porosity, higher soil enzyme activity (β-glucosidase) and more diversity within the nematode community. Compacted soils tended to be the most susceptible to soil borne diseases.

The challenge of the future is to improve the resilience and suppressiveness of vegetable-growing soils and enhance the sustainability of our intensive vegetable production systems. We need to know how various management practices alter soil physical, chemical and biological properties, and from a soil health perspective, understand their flow-on effects on soil borne diseases. Improved knowledge of soil ecology, together with the development of new techniques for monitoring the soil biota, provides opportunities to achieve this. One important need is to develop easy-to-use indicators to help growers and advisors assess the progress they are making during the early stages of system implementation.

References
Grain growers demand cereal varieties that provide high and stable yield. Part of achieving the release of such varieties is the incorporation of resistance and/or tolerance to soilborne diseases. The greater the resistance and/or tolerance to a range of diseases the greater the likely yield and yield stability across a range of environments.

This paper provides some direction to the pathology and pre-breeding community as to how they might work with commercial breeding programs in delivering the benefits of soilborne disease resistance and/or tolerance to the cereal industry.

The process of plant breeding involves four basic steps:

1. Prioritization of the importance of traits for target environments and products
2. Identification of useful genetic variation.
3. Recombination of that genetic variation.
4. Selection of individuals or lines with a favourable combination of these traits.

For a commercial plant breeder to develop cultivars with disease resistance and/or tolerance they must work with the pre-breeding and plant pathology sectors who can help prioritize the importance of each trait, identify useful genetic variation, describe the inheritance and linkage and also provide the tools to assist with the selection of individuals or lines with the desirable combination of genes expressed as traits.

The conundrum faced by a plant breeder is that there are many potential traits to target but the breeder must work within a finite resource. As the number of traits selected increase, the population size required to maintain genetic gain for each trait increases. For a plant breeder to target one additional trait that is controlled by a single dominant gene, the population size the breeder works with needs to double in order to maintain current progress with the addition of this new trait. If the inheritance of the trait is polygenic, recessive, partially dominant or of low penetrance, then the situation is worse. Alternatively if in the breeding process, population size is maintained at a static level, then the genetic gain for each extra trait will be compromised. Prioritization is a key to limiting selection to only the traits of importance for a target environment to allow maximum genetic gain for these traits.

Cereal cyst nematode (*Heterodera avenae*), once the most damaging disease of cereals in south eastern Australia has effectively been controlled through the development and widespread cultivation of varieties with resistance (Vanstone *et al.* 2008). The release of resistant cultivars followed 20 plus years of research by plant pathologists and pre-breeders in the identification and characterisation of sources of resistance, and the development of phenotypic and genotypic assays that were then utilised by plant breeders to progress resistance through the breeding programs and into the eventual release of new cultivars (Rathjen *et al.* 1998).

Unlike cereal cyst nematode, for which sources of resistance were identified, there are other diseases for which there has been little effective progress in breeding for resistance and/or tolerance. These include Rhizoctonia bare patch, (*Rhizoctonia solani*) and take-all (*Gaeumannomyces graminis*). For these diseases, the problem is a lack of genetic variation within the currently utilized gene pools. These diseases may be good examples of where alternative technologies including mutation breeding (within the gene pool), interspecific hybridization (closely related relatives), or transgenic methods (potentially from any species) may have to be deployed to find useful variation. Mutation breeding has been effective in developing herbicide tolerant cereals, interspecific hybridization has provided many of the major genes for rust resistance currently deployed and there are examples of insect resistance and herbicide tolerance derived from transgenic approaches.
The process of recombination is usually via sexual recombination with traditional pollination and generation advance to recombine the genes in novel ways and this will remain the major means of genetic recombination.

Efficient selection is the key to maximizing heritability and genetic gain within the available physical and economic resources, but is the most resource-consuming component of a plant breeding program. Technologies such as molecular markers enable direct selection of the plant’s genotype, thus avoiding the environmental component of the plant’s phenotype which in terms of selection is error, thus maximising heritability. However, for many traits, inheritance is complex or we do not have the tools to directly target the genotype, hence we must develop and utilize assays for traits that efficiently and effectively allow us to select desirable phenotypes. There are many good examples of accurate, reliable and cost effective assay systems that plant breeders use in selection for a range of diseases. Highlights in the selection of cereals for soilborne diseases include: development of molecular markers for cereal cyst nematode (*Heterodera avenae*) and root lesion nematode (*Pratylenchus spp.*). For foliar and stubble-borne diseases, recent examples include development of an assay for the Tsn1 gene (insensitivity to toxA), one of the toxins associated with yellow leaf spot (*Pyrenophora tritici repentis*) and stagonospora nodorum blotch (*Stagonospora nodorum*). There are also a number of examples of effective, high throughput bioassays, such as for rust (*Puccinia spp.*), cereal cyst nematode, and crown rot (*Fusarium spp.*). resistance, and boron and aluminium tolerance, but each of these can be improved to reduce cost, increase throughput and increase reliability to make them more valuable to plant breeders.

Clearly, resistance to soilborne diseases can be enhanced through close links between plant pathologists, pre-breeders and plant breeders. Prioritization of the relative importance of traits, identification of novel genetic variation, development of effective, cheap and reliable assays, plus an improved understanding of the inheritance of the traits by plant pathologists and pre-breeders are key for plant breeders to maximizing genetic gain to develop elite cereal varieties that are better able to resist the soilborne biotic stresses.

References
MICROBIAL SEQUESTRATION OF ORGANIC CARBON

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Agricultural soils rely on the properties of the topsoil for optimising productivity. Current management practices reduce organic carbon (OC) in soil. OC is crucial to many important functions. Restitution of OC content in topsoil must be a priority if we are to use topsoil sustainably. Addition of compost or green manure crops results in maintenance of OC indicating a concurrent increase in degradative processes. This paper proposes a model to explain the contribution of fungi to carbon sequestration in soil.

A comprehensive study of soil carbon in Germany indicated stable forms of OC were protected within aggregates (Kogel-Knabner et al. 2008). The most widely accepted model of soil aggregation (Tisdall and Oades 1982) indicates soil particles are held together by adhesive materials and enmeshed by hyphae and fine plant roots.

Our model specifically hypothesises the adhesion between particles by fungal polymers forming micro-aggregates, and the enmeshment of micro-aggregates to macro-aggregates by fungal hyphae. As the most stable OC is held within micro-aggregates, we further argue for the deposition of polyphenolic materials as the predominant form of stable carbon in micro-aggregates, and the protection of these polyphenolics from chemical and microbial oxidation by a variable cover of biofilm. Our model specifies the involvement of fungi. The mycelial form distributes fungi and anchors aggregates. In addition, energy from the roots of plants provides endophytic fungi with a competitive advantage over saprotrophic microbes in the oligotrophic soil matrix.

We focussed on arbuscular mycorrhizal fungi (AMF) as the basis of enmeshment. As AMF are rarely melanised, we additionally considered melanitic endophytic fungi as a source of polyphenolic materials. AMF, organic matter and host plants together developed aggregation and enhanced water characteristics of a massive material, mine spoil from the Hunter Valley, NSW. The carbon content of the micro-aggregate fraction increased. In addition, some saprotrophic and endophytic fungi increase soil aggregation, both by adhesion and enmeshment. Thirdly, some fungi express mucilage that coats aggregates.

In conclusion, our data support the hypothesis that soil fungi play a role in sequestering carbon in soil. Mine spoil was transformed to a material that resembled soil following the addition of plants, selected fungi and organic matter. These results indicate a process to restore degraded soil.

References
Molecular diagnostic tests to quantify soilborne pathogens have been available to Australian grain growers for more than a decade via Predicta B testing service (Ophel-Keller et al., 2008). Growers use the service to monitor pathogen levels in potentially high-risk rotations e.g. cereal on cereal, or to establish baseline pathogen levels where little information is available on cropping history e.g. new properties or leased paddocks.

Most management decisions to minimise losses from soilborne diseases need to be made before the crop is sown, so knowledge on pre-plant levels of soilborne pathogens is potentially valuable to growers. In the grains industry, consultants are best positioned to integrate information on pathogen levels with cropping programs of individual clients. So PreDicta B has been made available to growers only via consultants who have been trained to interpret results (Ophel-Keller et al. 2008). The most useful tests for growers are those where good strategies have been developed to manage disease risk, e.g. take-all can be managed by rotation or use of fungicides, cereal cyst nematode by rotation with non-host crops and/or by sowing resistant and tolerant cereal varieties.

Molecular diagnostic assays are useful tools to quantify soil-borne pathogens, because they can quantify multiple pathogens in DNA extracted from a single soil sample. While molecular diagnostics offer significant benefits to growers planning cropping programs, demand has been lower than market surveys indicated. A survey to identify barriers to adoption revealed that consultants use the service strategically and often extrapolate results to other paddocks with similar rotations.

A similar service is being trialled for potatoes, as part of the Australian Potato Research Program. Tests have been developed or validated for key pathogens of processing and fresh potatoes, including *Spongospora subterranea* (causal agent of powdery scab), *Streptomyces scabies* (common scab), *Meloidogyne fallax*, *Rhizoctonia solani* anastomosis groups 2-1 and 3, and *Colletotrichum coccodes* (black dot). Research to develop a number of these tests has been undertaken in the United Kingdom (Lees et al. 2002, Van de Graaf et al., 2003). In the potato industry, growers regularly lease new ground so DNA testing has a clear role in paddock selection where there may be little information about soil pathogen status. Pathogen load on seed tubers is a significant issue for the potato industry, and visual inspection of tubers may significantly underestimate pathogen status. Ultimately, it is envisioned that testing of seed and soil will allow seed to be matched with available paddocks e.g. avoid using infested seed in clean paddocks. Pre-plant testing might also lead to targeted fungicide application where there are few options for resistant varieties or rotation with break crops. Some of the tests developed for potato soilborne pathogens have broader applicability for horticultural industries e.g. root knot nematode (Stirling et al., 2003). Uptake of the technology in high-input, high-value horticulture may be more rapid than for broad-acre agriculture.

Uptake of quantitative DNA testing technology by the research community has been rapid. In addition to pathogens, tests have been developed for arbuscular mycorrhizal fungi (AMF) and *Trichoderma* species, while new research is underway to develop tests for selected genera of free-living nematodes to enable growers to monitor the biological status of soil and scientists to identify key taxonomic groups implicated in disease suppression. Tests have also been developed to quantify plant roots in soil (Mc Kay et al. 2008). Assays for wheat, barley and canola and key pasture species (lucerne, phalaris, ryegrass, subterranean clover, barley grass and silver grass) are capable of quantifying very small amounts of plant DNA in soil. Using ryegrass as a model, Riley et al. (2009) have shown that changes in plant
DNA in the soil are more rapid than changes in root dry weight, indicating that DNA assays are sensitive enough to detect changes in root growth and function.

The ability to use DNA assays to quantify soil borne pathogens, beneficial organisms and plant root growth in large numbers of samples is a powerful tool and should complement studies using metagenomic analysis. The latter can identify all taxonomic groups present in soil as well as genes encoding critical enzymatic pathways. These technologies may provide insights to underpin the development of rapid, cost-effective methodologies for growers to monitor soil biological status. Regardless of the technology used, the value for growers comes from the benefits achieved from the management decisions implemented, rather than the test result per se. This will determine the price that growers will be willing to pay.

References
Cullen DW, Lees AK, Toth IK and Duncan, JM (2002). Detection of *Colletotrichum coccodes* from soil and potato tubers by conventional and quantitative real-time PCR. Plant Pathology 51:281-292.
The Grains Research and Development Corporation (GRDC) has invested in soil biology RDE for more than 20 years, with two major investment initiatives over the last decade. The first Soil Biology Initiative (2002-2006), incorporating co-investments with Meat and Livestock Australia (Soil Biology in Pastures) and Land and Water Australia (Healthy Soils for Sustainable Farming; HSSF), significantly enhanced knowledge of the role of soil biology in soil health and plant productivity. The first initiative generated significant knowledge on regional soil biology issues that have been captured in conference proceedings, scientific papers, training modules and GRDC-commissioned reviews. Major RandD findings were summarised in the Australian Journal of Soil Research, Special Issue: Soil Biology in Australian Farming Systems (Murphy et al. 2006) and captured under three themes:

a) **Plant-microbe interactions:**
   - Productivity gains may come through manipulation of root growth, rhizosphere exudates and microbes (Watt et al. 2006)
   - Brassica crops stimulate soil mineral N accumulation. The magnitude of increase is site specific but ranges from 12 kg/ha (an underestimate) to 93 kg/ha (attributed to OM build up from preceding lucerne; Kirkegaard et al. 2006)

b) **Beneficial microbes**
   - The potential for non-symbiotic N₂ Fixation (NSNF) is significant in some cropping regions. NSNF contributes on average up to 40 kg/ha per year to the N status of soils in southern Australia; in regions with dry summers (WA, SA) estimates are low (10-15 kg/ha); in regions with summer rainfall (Qld), estimates are higher (32-38 kg/ha) (Gupta et al. 2006)
   - *Pantoea* and *Exiguobacterium* reduced Rhizoctonia disease in sterile soil by promoting root growth; (Barnett et al. 2006)

c) **Agricultural management (inputs and cropping systems)**
   - Impact of agricultural inputs on soil organisms is variable: Mineral fertilisers have limited direct effects and numerous indirect positive effects (increases in system productivity, crop residue return and soil organic matter) and indirect negative effects (soil acidification). Organic amendments (manure, compost, biosolids, humic substances) have a direct positive effect (provision of C) and an indirect positive effect by increasing plant growth and residue returns (provision of C). Microbial inoculant effects on biology appear small and transient. Herbicides have few significant effects and negative effects are common with insecticides and fungicides (Bünemann et al. 2006).
   - Liming at the recommended rate can influence beneficial microbes (eg N-fixers) in the rhizosphere (Nelson and Mele 2006)
   - Increased wheat yields (31%) are associated with retention of stubble (compared to burning). Stubble retention supported higher microbial biomass (34%) and respiration (61%), and greater enzyme activity (phosphatase and β glucosidase). Season (temperature) determined the impact of stubble treatment on N-processes (mineralisation, nitrification, immobilisation), with differences only at 30°C. Sampling time but not stubble treatment influenced community structure and function (Hoyle et al. 2006).
   - Stubble retention and reduced tillage leads to increases in earthworm abundance, measurable after at least 2 years of treatment imposition (Chan and Heenan 2006)
The biological status of grain growing soils in northern Australia is low compared to other land-uses. Management practices such as stubble retention and zero tillage produce relatively small benefits that are often confined to top 0.05 m of soil. Long fallowing reduced all soil biological parameters; breaking a long-fallow with short duration grain or brown manure crop moderated negative effects. Pasture leys produced consistent positive benefits. Use of inorganic P and N had minimal effects on soil biota (Bell et al 2006).

A fourth area, funded more recently, is focussed on monitoring the quality of cropping soils to enable better (regionally relevant) decision making. This involved the establishment of a soil quality database (http://soilquality.org.au) that contains data on the physical, chemical and biological properties of the major grain-growing soils in Western Australia. Growers can add their own test results to the database and compare their results with farms in their own catchment, area or region. The following are some of the indicators included: bulk density, water holding capacity, pH, EC, N, P, K, total C, labile C, microbial biomass C and inoculum levels for important soil-borne pathogens. For each indicator, a series of critical values have been applied relating to impacts on production and/or soil quality in general. This allows growers to quickly identify possible soil constraints to production and formulate management strategies to combat these issues. If more information is required, a series of fact sheets can be accessed that provide details of specific indicators and instructions on how to measure and interpret soil analysis results. Information on management practices that growers might use to overcome specific soil problems (e.g. compaction and water-logging) is also included.

A second $9 million initiative entitled Harnessing the biological potential of soils is now underway (2009-2014) and builds upon the substantial knowledge generated in the previous soil biology program. It is focused on three theme areas:

1) Monitoring soil quality for better decision making. This will extend the Soil Quality database outlined above to the eastern states and provide more detailed information on soil microbial and nematode functional groups. It will build upon the existing measures of microbial biomass and a suite of standard chemical, physical tests and measures of plant performance.

2) Management systems for enhanced nutrient availability (incorporating rhizosphere). This will focus on developing strategies to improve synchrony between biological N supply and plant demand. It will also include a study to quantify NSNF and the major players. A further focus will be on strategies for unlocking P that is fixed to soils.

3) Disease suppressive soils: traits and transferability. This will examine the application of emergent biotechnologies of metagenomics and metabolomics to develop a fingerprint for disease suppressiveness, focussing primarily on sites where suppression to Rhizoctonia has been established in southern Australia. Further studies will examine suppression of root lesion nematodes in northern Australia. Whilst soils suppressive to Rhizoctonia have been identified in south-eastern Australia, efforts will also be made to identify whether this phenomenon occurs in cropping zones in Western Australia.

This presentation will summarise major findings from the GRDC investment in soils and showcase the new projects in each theme area, emphasizing how these will contribute to enhanced plant productivity and other important ecosystem goods and services.

References:
Papers cited in this abstract can be found in:
POTENTIAL APPLICATIONS OF SOIL MICROBIAL METAGENOMICS

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The soil metagenome is defined as the collective genomes (bacterial, archaean, fungal and viral) in or recovered from soil (Handelsman et al. 1998; Daniel 2004; 2005). Soil metagenomics is also described as both a set of research techniques and a research field; the first context recognizing the need for computational methods to maximize understanding and the second recognizing that the focus is on the genes in the community rather than on individual organisms (Handelsman et al. 2007). The first context has received considerable attention in the literature reflecting the myriad technical challenges associated with this youthful field. These challenges include the need for single cell sequencing of the unculturable majority, the removal of the annotation bottlenecks, coordination of sequencing efforts and consensus on selection of model ecosystems (Handelsman et al. 2007; Riley and Buckley 2009; DeAngelis et al. 2010). As these challenges are resolved, the focus will move increasingly towards applications for soil metagenomics in diverse industries including agriculture, environment, bioenergy, pharma and manufacturing.

Soil metagenomics addresses a serious shortcoming of conventional soil microbiology: the inability to identify the vast majority (99%) of soil organisms and the functions they perform (Keller and Zengler 2004; Xu 2006; Chen et al. 2008). It is providing an unprecedented view of the taxonomic diversity, metabolic potential and ecological role of soil microbial communities enabling powerful resolution of the multiple ways in which soil microbes can benefit society. For example, in agriculture the potential benefits can be measured in three interrelated ways; i) the provision of transformational knowledge of soil processes of relevance to nutrient and disease management ii) the capacity to measure the impacts and risks associated with land-use change and soil management of soil microbial communities iii) new products for multiple purposes such as plant growth under suboptimal conditions, for biocontrol of disease, for bioremediation of pesticides and for the development of biofuels. Table 1 provides a summary of the ways in which soil metagenomics is being applied to generate outputs for a range of industries.

This presentation will review the multiple ways in which soil metagenomics is being applied to both old and new problems. It will focus particularly on the recent efforts to examine agricultural soils in Australia and explore how this new knowledge will shed light on existing problems associated with disease suppression and nutrient supply functions. It will conclude with two key messages. The first is that soil metagenomics will, as Jo Handelsman said ‘be the mother of a paradigm shift’ by principally providing knowledge at the aggregated community level rather than at the single species level (Handelsman et al. 2007). The second is that soil metagenomics is but one tool in an ever-expanding tool-box; the real cleverness will be in the integration of new and classic technologies, in the planning and application of robust informatics approaches and the merging of non-traditional disciplines to design both exploratory and hypothesis driven R&D activities that address critical societal problems (Oremland et al. 2005; Handelsman et al. 2007)
### Table 1: Examples of soil metagenomic contributions to a range of industry applications since 2000

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<thead>
<tr>
<th>Industry</th>
<th>Application</th>
<th>Specific Information</th>
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<tr>
<td>Agriculture</td>
<td>Plant growth promotion</td>
<td>Potential uses for selection and tracking</td>
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<td>Plant disease control: toxins, communication molecules, enzymes</td>
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<td>Biotoxins in symbiotic metagenomes (eg bacterial endosymbiont of the fungus, <em>Rhizopus</em>)</td>
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<td>Novel communication (HSL) molecules</td>
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<td>Chitinases in actinobacteria for fungal disease control</td>
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<td>Suppressive soils</td>
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<td>Plant breeding</td>
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<td>Lysine racemase gene as a selective marker in transgenics</td>
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<td>Glyphosate insensitive (synthase) gene</td>
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<td>Novel communication (HSL) molecules</td>
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<td>Nutrient</td>
<td>Ammonia-oxidizing archaea (AOA) are more abundant in many soils than bacteria</td>
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<td>Bioremediation</td>
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<td>Pyrethroid insecticide. hydrolyzing esterase gene</td>
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<td>Environment</td>
<td>C sequestration (climate change)</td>
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<td>Impact of deep ocean subsurface CO₂ geo-sequestration on methane production</td>
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<td>Management of C-rich peatlands that are rich in methonotrophs, dominated by Methylocystis-related species.</td>
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<td>Bioremediation</td>
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<td>Benzoate 1,2-dioxygenase to degrade contaminants</td>
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<td>Energy</td>
<td>Biofuels</td>
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<td>Cellulosic and lignosolic enzymes</td>
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<td>Pharmaceutical</td>
<td>Human disease management</td>
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<td>New enzymes and bioactive molecules (eg antibiotics)</td>
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<td>Manufacturing</td>
<td>Biocatalysts</td>
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<td>Enzymes from extreme environments; low temperature (Antarctic soils), high salinity (sediments)</td>
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### References


By 2030, world population is expected to increase to 8 billion and world wheat (*Triticum aestivum*) production to increase from 584 million tonnes (1995-1999 average) to 860 million tonnes (Marathee and McPherson 2001). The world wheat deficit during these three decades is expected to rise by 2.5 times, particularly in the developing world, where 84% of the population increase is expected and where wheat is a staple. To compensate for the additional demand for wheat, methods must be employed to minimise yield production constraints. Soil Borne Pathogens (SBPs) including the Dryland Root Rot and Cereal Nematodes are one of the ‘unseen’ enemies causing economic yield loss in many parts of the world where cereals are the predominant cropping system and the crop is grown under sub-optimal growing conditions, particularly drought/moisture stress. In these systems the option to use crop rotation with non-hosts is limited, and therefore one of the most cost effective, safe and logical management options is the use of genetic host resistance whereby the inoculum of these SBPs can be reduced below economically damaging thresholds. Since it is not uncommon to find more than one SBP in the soil at one time, these pathogens should be considered as a soil complex.

CIMMYT International under the ICWIP program, in collaboration with Turkey and several other National Agricultural Research Programs, has been working on the importance and control of SBPs in West Asia, North Africa, China and Northern India since 2002. Several advanced research Institutions in Australia, Europe and America have also been involved.

Work in Turkey has focussed on the Crown Rot (CR) species *F. culmorum*, whereas in many other regions this and a closely related species *F. pseudograminearum* have been reported and researched. CR causes significant economic losses, particularly in rainfed wheat conditions in many regions, including West Asia, North Africa, Latin and North America, Europe and Australia (Chakraborty et al. 2006). Similarly, several species of the two important cereal nematodes, Cereal Cyst Nematode (CCN- *Heterodera* spp.) and Root Lesion Nematode (RLN *Pratylenchus* spp.) have been reported to have a global distribution and be economically important, particularly under drought conditions (Nicol and Rivoal 2008). The key research objectives and their outputs between CIMMYT and the Turkey Ministry of Agriculture and Rural Affairs since 2002 are summarised below, with key references and regional relationships.

i) **Surveys** to understand the importance and distribution of SBPs have confirmed their widespread distribution, particularly in the rainfed wheat systems of West Asia and North Africa.

ii) **Yield loss and population dynamics** studies with key SBPs have that significant economic losses are occurring on wheat in many counties and regions, particularly under rainfed post anthesis drought stress.

iii) **Identification of genetic host resistance against SBPs** has commenced, with the emphasis on the identification of genetic resistance for multiple SBPs. To date, more than 15 sources of wheat in adapted backgrounds have been shown to have resistance against multiple SBPs (Nicol 1007, 2010). In addition, more than 20 other sources of resistance to individual SBPs have been identified.
iv) **Integrated management options for SBPs with methods other than genetic resistance** have been trialled. Various resource conserving technologies (RCTs) have been tested, including host rotation and reduced tillage for cereal nematodes.

v) **Molecular tools** for pathogen diagnostics and to identify genetic regions of resistance in plant populations are being used. Known and useful markers for Marker Assisted Selection have been applied where appropriate.

vi) **Training and capacity building** of National Program Scientists in the region through postgraduate training with local Turkish Universities has been an important priority. Additionally, several specific, targeted and intensive regional and international Master training courses have focussed on the SBPs. International Workshops for specific SBPs such as the 1st International Cereal Cyst Nematode Workshop (Riley et al. 2009), have also been held. These courses and meetings have had a significant impact on human capacity development by enhancing awareness and knowledge of SBPs (Centre for International Economics 2009).

Significant progress is being made in understanding SBPs in this region and providing management options. The approaches used will be briefly reviewed and key reference materials demonstrated.

**References**


Nicol et al. (2010). Adapted spring and winter wheat with resistance against multiple soil borne pathogens (Cereal Nematodes – *Heterodera filipjevi* and *Pratylenchus spp.* and Crown Rot – *Fusarium culmorum*) targeted for the rainfed wheat production systems. In *this proceedings*.


**Acknowledgements**

Key donors who have supported this work include Grains Research Development Corporation (Australia), The Crawford Foundation (Australia), Australian Centre for International Agricultural Research, USAID Linkage Funds, Syngenta, Sp-IPM – System wide program Integrated Pest Management, The Kirkhouse Trust United Kingdom and the management and staff of the National Programs of North Africa (Morocco, Tunisia and Algeria), West Asia (Turkey, Iran), China and India.
Phytophthora comprises a genus of devastating plant pathogenic microorganisms. They have been responsible for some of the most extensive disease epidemics and continue to pose problems with agricultural and horticultural crops worldwide. Taxonomically Phytophthora are classified with the Stramenophiles and are quite distant from the true fungi. Unlike the fungi they have diploid vegetative cells and cellulose cell walls (Hardham 2005). Their closest relatives are the alveotates, such as *Plasmodium*, *Toxoplasma* and *Cryptosporidium*.

Genetic variation arises by mating between A1 and A2 mating types although some species are self fertile and do not require the opposite mating type. Importation of strains of the opposite mating type can stimulate a burst of sexual recombination leading to the emergence of new genotypes. The importation of new genotypes of the potato late blight pathogen *P. infestans* into the USA from Mexico in the late 1980’s led to an almost complete change in the genetic structure of the resident *P. infestans* population (Goodwin et al. 1998). New species also arise by hybridization between two species. *P. alni* a new species that is causing extensive damage to stands of Alder trees throughout Europe is a hybrid of *P. cambivora* and an unknown taxon similar to *P. fragariae* (Brasier et al. 1999). New species are continually being described and may represent new hybrid species (Man In't Veld et al. 2007).

Traditionally detection is by baiting of infested soil, or by plating diseased tissue on selective agar (O'Brien et al. 2009). More recently a number of DNA tests have been developed for detection (O'Brien et al. 2009). Progress is being made towards the development of on-site DNA identification tests. On-site antibody tests have been developed and are used extensively in the UK to screen nurseries for *Phytophthora* pathogens.

**References**


IMPORTANCE OF SOIL ORGANIC MATTER TO SOIL HEALTH AND DISEASE SUPPRESSION IN VEGETABLE CROPS

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Over the past decade, the need to find new sustainable practices to control soilborne plant diseases has increased dramatically. Regulations on pesticide use worldwide, particularly fumigant chemicals, are becoming increasingly prohibitive owing to increased concerns of their effects on the environment, human health and food safety. This is forcing industries to consider and implement other more sustainable practices to control soilborne disease. Industries that were once highly dependent on pesticide use, such as the vegetable industry, are starting to realise that greater ecological balance is required in their farming systems, but they are unclear how to measure or value the impact of sustainable practices. It is also unclear which practices will provide the profit margins necessary to economically sustain farming into the future. This is especially true in the highly cultivated vegetable crop production systems predominantly found in Australia where normal crop management severely disrupts ecological balance by use of a large amount of synthetic and other inputs (e.g. tillage, fertilizers, pesticides, water).

In contrast, many broad acre industries (e.g. cotton, grains, sugar cane) use more sustainable cropping practices. Greater use is made of crop rotations for disease control, varieties with resistance to disease are common and conservation tillage is used to control specific diseases and improve productivity. Additionally, greater advances have been made to improve soil health and disease suppression (Weller et al., 2002; Mazzola, 2004; Janvier et al., 2007). Benefits are especially seen in systems which are cultivated less frequently, as this allows for the ecological balance and resilience to be maintained and competition by microbial saprophytes to be established. Consequently, in these systems, the investment into preservation of organic matter and organic carbon has the added benefit of higher microbial diversity and activity, and therefore disease suppression. However, does the same hold true for highly disrupted vegetable crop production systems? What is the role for using organic amendments to improve disease control and soil health in these systems? And, of these amendments, which provide increased structural diversity in soil microbial populations and the necessary ecology to suppress disease?

In reviewing the literature, it is clear that some organic products decrease soilborne pathogens, either by producing toxins during the breakdown of organic products or by changes in soil microflora inducing suppression. For instance, hardwood composts have reduced *Pythium* and *Phytophthora* spp. (Hoitink and Fahy, 1986), high C:N ratio organic amendments, ie. low N enriched systems (e.g. sawdust, grass hay and sugar cane trash) have reduced populations of specific nematodes through biological shifts (Stirling et al., 2005), whereas high nitrogen amendments have controlled *Streptomyces* spp., *Verticillium* spp, and nematodes through changes in nitrogen form and other chemical shifts (Lazarovits, 2001, Oka 2010). Biofumigant crops, artificial stimulants and the use of anaerobic fermentation of residues are also methods used successfully to control diseases (Kirkegaard and Matthiessen, 2006; Lamers et al., 2009). Less frequently, however, other researchers have shown that organic debris, particularly undecomposed residues, can increase disease (Termorshuizen et al., 2006). The common understanding in all examples above is that generally all organic amendments increase labile carbon, microbial activity and diversity, and that organic amendments are good for soil ecology and generally for disease control. However, these examples show that different mechanisms will be responsible with different organic products and different host/pathogen systems.

To further elucidate the effects of organic amendments, a project funded by Horticulture Australia Limited and Ausveg in the temperate vegetable industry has been
conducted on the relationship between soil health and disease suppression. This project evaluated over 35 indicators and tests for physical, chemical and biological soil health and related them to disease suppression. It also conducted short and long term studies to evaluate the impact of organic amendments on crop health and yields compared to the growers’ standard fertilizer and pesticide programs. In particular, the trials evaluated whether, under intensive cropping in southern Australia involving many cultivations, organic amendments could build soil carbon and lead to increased benefits in soil health and disease suppression. The trials showed that regular inputs of organic matter (>5-10t C/ha per crop) in comparison to the standard grower practice without organic amendments, generally increased broccoli yields in both the short and long term trials (Porter et al. 2010). The organic products, however, differed markedly in their ability to decrease disease. In the field trials, three organic amendments (ie. composted chicken manure, composted green waste and lignite) promoted clubroot caused by Plasmodiophora brassicae, and one (ie. silage) decreased it. In pot studies, lignite, composted green waste and a humate tended to decrease damping off caused by Rhizoctonia solani (Guijarro et al., 2010). These differences in control were considered to be related to the C:N ratio, the composition and breakdown rate of the parent material and the effect of the amendment on soil pH. The lack of control of clubroot was directly correlated with the decrease in pH caused by the organic treatments. In spite of higher disease, chicken manures gave higher and more profitable yields indicating that there were additional soil health and plant productivity benefits beyond those of the other products.

The trials also showed that the organic amendments generally had a positive benefit on many soil health characteristics (ie chemical, biological and physical analyses) particularly improving microbial activity, carbon levels, water content and other chemical characteristics.

Despite the list of positive benefits from the organic amendments, the composted chicken manure was shown to emit a high level of N₂O (an important greenhouse gas), considerably higher than that from the synthetic fertilizer and other organic treatments. Present trials are aimed at finding methods which mitigate this effect to ensure that any future use of organic treatments minimises the impact on the environment as well as providing the ecological balance that should be strived for in future vegetable production systems.

References


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UNDERSTANDING VARIABILITY IN BIOCONTROL SYSTEMS

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Trichoderma biocontrol of soil-borne plant diseases has been successful but variability in efficacy across diverse cropping systems is still the key constraint to widespread uptake of this technology. Limited understanding of the complex biotic and abiotic relationships operating in the soil environment is considered the main reason for our inability to effectively ‘manage’ Trichoderma biocontrol (Kredics et al. 2003). This paper presents a summary of research conducted over the last 8 years investigating the complex influences of a range of soil factors on two commercial Trichoderma isolates, T. hamatum 6SR4 the active ingredient of Trichodry™ 6S and Trichoflow™ 6S used to control Sclerotinia lettuce drop disease (Rabeendran et al. 2006) and T. atroviride C52, the active ingredient of Tenet®, used to control onion white rot disease (McLean et al. 2005).

Previous studies using strain specific markers to monitor Trichoderma populations in the soil showed that a population threshold of $10^5$-10$^6$ cfu/g soil was necessary for each Trichoderma isolate to be able to give effective disease control. This threshold was used as the baseline for determining the stimulatory/inhibitory effects of selected soil parameters. Intensive laboratory based studies examined the effect of micro and macro nutrient sources, C:N ratio and pH on spore germination, mycelial growth, sporulation pattern and expression of a number of key genes implicated in biocontrol. Results showed that nitrogen source and pH were the key factors which influenced the growth behaviour of each isolate. For example, small changes in pH (0.4 units) induced significant changes in growth pattern and sporulation of both isolates, but these changes occurred within different pH ranges (Steyaert et al. 2010). 0.2% urea significantly inhibited spore germination and mycelial growth of T. atroviride C52 but not T. hamatum 6SR4. In contrast, inorganic nitrogen was shown to significantly repress the expression of chit$^42$ and prb1, genes implicated in mycoparasitism by T. hamatum but this response was less evident for T. atroviride C52. Soil type was also shown to differentially affect biocontrol activity. T. atroviride C52 performed best in volcanic soils whereas T. hamatum performed best in clay soils and both isolates performed poorly in sandy soil.

It is well reported that Trichoderma species are compatible with organic amendments such as composts, green manures and bark and it is common practice to use these substrates as carriers for commercial products. However, our research has shown that such amendments are not necessarily beneficial in all circumstances. For example, T. atroviride C52 applied as a pellet formulation into the soil at planting was highly compatible with poultry manure and spent mushroom compost. Both treatments significantly enhanced the colonisation of the BCA in the soil over a 2 month period and resulted in good disease control. However, this effect was only found in volcanic and clay soils but not sandy soils where Trichoderma levels were significantly reduced by amendment with poultry manure and disease control was compromised. In contrast, T. hamatum 6SR4 applied to soil in the same manner was compatible with poultry manure in a sandy soil but not clay and volcanic soils. A similar differential effect was observed for fertiliser application. Urea based fertilisers stimulated T. atroviride C52 growth in some soil types but not others and this differed to the pattern of response shown by T. hamatum 6SR4 for the same soil types.

Although specific environmental parameters have been shown to influence the growth and development of Trichoderma strains, two key biological attributes, competitive saprophytic ability and tolerance to abiotic stress, were found to have the greatest influence on biocontrol performance. Ability to germinate and proliferate quickly in the presence of competing microbes under conditions of environmental stress was shown to be an absolute requirement for biocontrol strains to be effective in the soil environment. We have used this
information to develop more targeted screening programmes that select microbes on the basis of these priority attributes.

In a commercial field setting, both biocontrol products are used as part of an integrated disease management system. Compatibility of the biocontrol products with other crop management systems is therefore an important factor that must be addressed. Our research has shown that *T. atroviride* C52, when applied to the planting furrow, is compatible with procymidine, benomyl and captan but not thiram when applied as onion seed treatments. It is also compatible with the majority of other fungicides applied to the onion crop to protect against foliar diseases such as *Botrytis* and downy mildew (Stewart and McLean 2004). The product can be integrated with the use of the germination stimulant diallyl disulphide (DADS) as long as the *Trichoderma* product is applied at least 2 weeks after DADS application. The product is not compatible with the use of nitrogen fertilisers due to the sensitivity of the *Trichoderma* mycelium to high N, thus, care must be taken to separate fertilizer application from that of the biocontrol product.

From these studies, we have identified pH and N status as significant influencing factors on biocontrol performance and prioritised the key biological attributes (competitive saprophytic ability, tolerance to abiotic stress) required by *Trichoderma* biocontrol agents to provide effective and consistent biocontrol. Information gained on the effects of soil factors on *Trichoderma* growth and development has provided some explanation for the variability in biocontrol observed in the field. This information is being used to provide better recommendations for use of the commercial products, for example, with respect to their expected efficacy in different soil types and their compatibility with various organic amendments and fertiliser applications.

References
AN INTERNATIONAL PERSPECTIVE ON BREEDING FOR RESISTANCE TO
SOIL BORNE PATHOGENS

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The extent and impact of soil borne pathogens on the wheat producing areas of the world is significantly underestimated. Many farmers and researchers are unaware of the problem and poor crop performance is often attributed to drought or other constraints. Plant breeders need to understand the extent of the problem in their target environments if appropriately resistant cultivars are to be developed and deployed. An international adaptation trial was assembled at CIMMYT in collaboration with Australian partners and distributed across the wheat growing areas of the developing world and Australia between 2001 and 2004. This trial contained sets of lines with similar genetic backgrounds but contrasted in their yield response to various soil borne constraints such as cereal cyst nematode (Heterodera spp.), root lesion nematode (Pratylenchus thornei and P. neglectus) and dryland root rot (Fusarium sp.). The aim was to identify possible soil borne limitations based on the yield performance of contrasting pairs or groups of germplasm. Results indicate that root rots, nematodes and micronutrient imbalances are likely to limit production in many environments.

On the basis of these findings, the results of soil surveys and local observations of disease expression, plant breeders can deploy resistance genes in appropriate genetic backgrounds. The CIMMYT wheat breeding program has used a combination of molecular markers and screening in ‘hot spot’ environments such as Turkey to develop and validate resistant germplasm. Many new sources of resistance have been found and distributed to scientists in North Africa, West Asia, China, India and Australia. In an exciting development for plant breeders, some sources have confirmed resistance to more than one pathogen.
Biochar is of interest to soil and climate scientists because of its potential to increase soil C long-term. It is derived from biological materials which have been pyrolysed (heated to temperatures exceeding ca. 450°C under low O₂), essentially stabilizing C into condensed aromatic structures. During production in engineered processes, renewable energy is produced which can off-set emissions from fossil fuels. It is well understood that the nature and function of soil microbial communities change in response to organic matter addition to soil (Thies and Grossman 2006). Biochars are less well studied, but recent work has shown potential to change the physical and chemical properties of soils and therefore influence soil microbial communities. This presentation reviews the science of pyrolysis and biochar, and discusses some unique aspects that may be applicable to managing soilborne diseases.

**Biochar influences soil physical and chemical conditions**

The application of biochar to soil influences a wide range of soil limitations including low pH and high available Al (Van Zwieten et al. 2010), soil structure and nutrient availability (Chan et al., 2007), bioavailability of organic (Yu et al. 2009) and inorganic contaminants (Hua et al., 2009), CEC and nutrient retention (Major et al. 2009), and organic matter decline (Lehmann et al., 2006). Biochars have a highly porous structure with surface areas sometimes exceeding 1000m²/g (Downie et al., 2009). Like activated charcoal, they adsorb organics, nutrients and gases, and are likely to provide habitats for bacteria, actinomycetes and fungi (Thies and Rillig, 2009). Increases in water holding capacity following biochar application to soil have been well established (Pietikainen et al., 2000), and this may influence soil microbial populations and population flux in wetting/drying cycles. Data from our studies have shown that biochars influence availability of N, P, K and micronutrients in soil. The application of a low nutrient biochar derived from timber increased the retention of N in soil and uptake of N into crop biomass (Steiner et al., 2008). Similarly, the application of charcoal derived from bamboo into a sludge composting system was shown to provide significant increases in N retention in the compost (Hua et al., 2009). Increased fertility of soil resulting from biochar application is likely to increase crop vigor, and thus may enhance disease tolerance.

**Biochar amendment changes soil microbiology**

Biochar has been shown to increase biological N₂ fixation (BNF) of *Phaseolus vulgaris* (Rondon et al., 2007). This study reported a BNF increase of 49 and 78% with 30 and 60g/kg biochar additions respectively, largely due to greater boron and molybdenum availability. Recent work in fababean (*Vicia faba*) in northern NSW has demonstrated N return to soil from BNF increased from 18kg/ha in standard farmer practice to around 60kg/ha with biochar amendments. Concomitant increases in biomass and bean yield were noted. Other studies have reported increased microbial activity with biochar amendments (Steiner et al., 2008; Kolb et al., 2009). Pietikainen et al. (2000) reported adsorption of 42% of dissolved organic carbon into biochar from a litter extract, which provided substrate for microbial growth. Recent studies have reported increased N mineralisation and nitrification through biological processes with biochar amendment in forest soils (DeLuca et al., 2006). In agricultural soils N mineralisation and nitrification may be reduced by biochar addition due to either N immobilisation or a decline in mineralisation of organic N (Lehmann et al., 2006). Recent work has shown biochar increases plant growth and subsequently increases nutrient availability to mycorrhizal fungi (Makoto et al., 2010).
Biochar reduces the risk of introducing pathogens
The transport and return of untreated organic matter to soil increases the risk of introducing human, animal and plant pathogens. However, opportunities exist to eliminate this pathogenic load through pyrolysis, which essentially sterilizes the material at high temperatures. This process may have a role in reducing disease loadings in organic products, whilst maintaining organic matter inputs into soil.

Impacts on soilborne diseases
Although there is a paucity of published data on the effects of biochar on soilborne pathogens, evidence is mounting that control of certain pathogens may be possible. The addition of biochar (0.32, 1.60 and 3.20 % (w/w)) to asparagus soils infested with Fusarium root rot pathogens increased asparagus plant weights and reduced Fusarium root rot disease (Elmer et al, 2010). Further, Matsubara et al (2002) (cited in Thies and Rillig 2009) have shown that biochar inoculated with AM fungi are effective in reducing Fusarium root rot disease in asparagus. A study of bacterial wilt suppression in tomatoes found that municipal waste biochar reduced the incidence of disease in Ralstonia solanacearum infested soil (Nerome et al, 2005). The mechanism of disease suppression was attributed to the presence of calcium compounds, as well as improvements in the physical, chemical and biological characteristics of the soil. Likewise, Ogawa (2009) describes the use of biochars and biochar amended composts in reducing bacterial and fungal soilborne diseases.

References
Soilborne diseases of cereals have some common features that make developing more resistant varieties particularly problematic. Resistance to these pathogens is partial at best, with the most effective sources often having multigenic resistance based on minor genes that are difficult to investigate and track in breeding programs. Compared with most foliar pathogens, screening lines for resistance to soilborne pathogens also requires time consuming and costly procedures and produces data which come with high standard error terms. On the other hand most soilborne pathogens share an absence of variation for virulence on the known resistance sources. This is a significant advantage for developing breeding strategies once suitable resistance donors have been identified.

An exception to the above generalisations is cereal cyst nematode (CCN). Resistance to this pathogen is controlled by major genes that can be reliably tracked through screening procedures and molecular markers are available and proven. Internationally, the pathogen also occurs as different species and biotypes carrying different virulence factors, although this is not the case in Australia where just a single pathotype of one species has been identified.

Many different sources of partial resistance have been identified for crown rot and loci (QTL) have been identified for a few of them (Wallwork et al. 2004, Bovill et al. 2010). Diverse breeding and pre-breeding strategies have been developed to transfer some of these resistances into elite germplasm although molecular markers have yet to be fully validated.

A smaller number of useful sources of resistance for root lesion nematodes have been identified. Resistance QTL have been identified for both Pratylenchus neglectus and P. thornei and one, Rlnn1, for P. neglectus resistance is being used in breeding programs now that reliable markers have been developed and validated.

Although some sources of partial resistance have been identified for common root rot, resistance to this pathogen has not been a priority for breeding programs so little or no breeding or pre-breeding activity is currently taking place.

Useful resistance for take-all or Rhizoctonia has yet to be identified in adapted germplasm although sources of resistance have been identified to both pathogens in distantly related grass species. Efforts to transfer these into elite germplasm are in abeyance. Resistance to Rhizoctonia has recently been reported in mutagenized wheat plants in the USA (Okubara et al. 2009) although field trials using these plants have not shown the resistance under farm conditions.

Progress, or lack of, in identifying variation to these pathogens will be covered in the presentation along with strategies for transferring resistance to wheat and barley.

References
INTRODUCTION

In macadamia, diseases caused by Phytophthora are capable of reducing vigour, productivity and may cause complete tree death. *P. cinnamomi* has been reported to cause trunk canker, root rot and quick decline in macadamia [1, 2]. Pathogen infection in macadamia is not well understood as infection processes leading to trunk canker, root necrosis and root rot diseases differ. In most phytophthora diseases, the presence of free water in the soil is critical for the dissemination of zoospores, and infection of feeder roots is enhanced by root exudates which attract the zoospores. Infection process of the macadamia cluster root system is unclear. Unlike typical fine roots, cluster roots are temporary, continually replaced and exudates are released from cluster roots for only a brief period [3].

There are varying reports concerning the importance of rootlet necrosis in macadamia [1, 4]. Environmental factors such as moisture content, temperature, organic matter and drainage affect *Phytophthora* infection of plant tissues. The effects of these factors on macadamia phytophthora diseases are relatively unknown. This study provides information on field observations of phytophthora diseases in macadamia orchards and the results of field and laboratory trials on *Phytophthora* infection on macadamia.

MATERIALS AND METHODS

**Effect of Phytophthora on macadamia roots**

In order to determine if *P. cinnamomi* causes root necrosis or root rot in macadamia, roots of seedlings of three macadamia varieties (H2, 508 and 781) produced from germinated seeds (nuts) on pasteurised sandy soil in the glasshouse, were immersed in water in cups containing either *Phytophthora*-infested soil or autoclaved soil for 6 weeks. The treated plants were kept under 12hr fluorescent light at 25°C. The roots of each treatment were observed for rot every week.

**Effect of soil health on macadamia**

Pre-germinated and fresh seeds of varieties H2, 246 and A4 were planted in pots containing *Phytophthora*-infested soil (PS), pasteurised potting mix (PM) and PM mixed at ratios 1:1 and 1:2. The number and height of seedlings that emerged, total leaf area, stem diameter, seedlings and root weights were recorded for each treatment after 5 months. Grafted trees of H2 rootstock for varieties 816 and 842 were planted in *Phytophthora*-infested field of rich, deep clay-loam soil. At planting, the trees were either planted in holes with Ridomil® Gold 25G and bark painted with phosphorous acid or planted in untreated soil. Performance of the trees was evaluated every 6 months for 2 years.

**Effects of plant health on Phytophthora infection.**

In order to determine the effect of plant health on *Phytophthora* infection, grafted trees of variety 816 with H2 rootstock in potting bags were bark-inoculated with *P. cinnamomi* and treated at varying times with phosphorous acid and/or fertilizer (Osmocote®). Presence and extent of stem canker, tree height and visual canopy rating were recorded at 4, 8 and 20 weeks after the last treatment was applied.

**RESULTS**

Roots of all seedlings immersed in water containing *Phytophthora* or clean soil did not show any root necrosis and rot during the observation period. Presence of *Phytophthora* in soil significantly reduced seedling growth, including root weight (Fig. 1). However, visual (above-ground observation) performance of trees planted in *Phytophthora*-infested but fertile soil without any treatments was similar to those planted with Ridomil fungicide and bark painted with phosphorous acid. Results showed that more severe stem canker occurred in tree infected before phosphorous acid was applied compared to trees that received phosphorous acid and/or fertilizer before inoculation.

**DISCUSSION**

Field observations showed that macadamia trees infected with *Phytophthora* showed various symptoms; trunk canker, sparse root network and tree decline with poor canopy volume and yellowing of leaves. Most diseased trees were in areas with a poor soil profile, sub optimal nutrition and had been under constant stress. This study shows that *Phytophthora* causes stem canker easily in macadamia after inoculation but little, if any effect is observed directly on the roots in the form of necrosis or rot. Both tree and soil health appears to influence susceptibility of macadamia to *Phytophthora*. A more sustainable management of phytophthora diseases in macadamia can be achieved through adequate management of soil structure and soil nutrition status that will invariably results in healthier plant.

**REFERENCES**

USE OF TELONE C35 TO REDUCE SOILBORNE RHIZOCTONIA INOCULUM FOR MANAGEMENT OF ONION STUNT

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INTRODUCTION
Scattered patches of stunted growth are widespread in onion crops of the Mallee region in South Australia (SA). Stunted onions have reduced bulb size, and in severe cases, plants are not economically harvestable.

While a number of *Rhizoctonia* anastomosis groups are pathogenic to onions, it is primarily *Rhizoctonia solani* AG 8 that is associated with the problem in Mallee onion production sites (1). The aim of this study was to determine the effect of the soil fumigant Telone C35 (35% chloropicrin and 61% di-chloropropene, Dow AgroSciences) on levels of *Rhizoctonia* in the soil, to determine its suitability as a management tool.

MATERIALS AND METHODS

Field Trial Telone C35 was injected to a commercial onion pivot on 27th August 2009 at rates of 150L/ha, 200L/ha (label rate) and 250 L/ha. Each rate was injected approximately 20cm deep in three plots each 260m long, roller pressed and lightly irrigated to seal the soil surface. A barley nurse crop was sown 19 days after fumigation (DAF) while Onion cv. Patrick was sown 34 DAF. Two soil samples (50 cores 1cm x 10cm) were taken from each plot to assess levels of *R. solani* AG 8 (SARDI Root Disease Testing Service) prior to fumigation and at 14 and 88 DAF. Onion stunting (seedlings < 50% normal leaf height) was assessed 60 days after sowing (94 DAF).

Soil core bioassay Seven soil cores (7.5cm x 12cm) were taken 102 DAF from either normal or stunted areas in both fumigated and non-fumigated areas of the crop. Cores were left to stabilise for two weeks (15ºC) at which time they were assessed for their level of *Rhizoctonia* infestation using toothpick baiting. The level of *Rhizoctonia* present in the soil is expressed as % toothpick area showing colony growth when re-plated onto semi-selective growth medium. Cores were sown with five Onion cv. Patrick seeds per core pre-germinated for three days at 25ºC. Seedlings were grown at 15ºC under a 12 hour day/night regime. After 6 weeks shoot and root dry weight was recorded.

RESULTS

Field Trial Soil sampling 14 DAF showed that Telone C35 reduced *R. solani* AG 8 at all rates (Fig. 1). By 88 DAF, negligible levels of *R. solani* were detected in all fumigated plots (mean value of 0.5 pg DNA/g soil across all rates), while non-fumigated plots had mean levels of 12 pg DNA/g soil at this time (data not shown).

With Telone C35 application, the incidence of stunted seedlings was increased (200 and 250L/ha) and seedling emergence was reduced at all rates (Fig. 2).

Acknowledgements SA Rural Agencies Pty Ltd, Wingfield, SA for providing Telone C35 fumigant and soil injection rig.

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A DESCRIPTIVE MODEL FOR IMPROVED MANAGEMENT OF CROWN ROT OF WHEAT

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INTRODUCTION
Crown rot caused by *Fusarium pseudograminearum* remains a persistent problem in wheat production despite decades of research on its management. One possible reason for this is the lack of an accessible framework for understanding the effects of rotation, resistance and other practices on populations of the pathogen in the medium to long term. A simple descriptive model for the disease would allow growers and researchers to appreciate the relative effectiveness of different management strategies, and aid planning of cropping sequences. This paper describes the development of such a model for bread wheat in the northern grains region.

METHODS AND RESULTS
Forecasting incidence In successive wheat crops, incidence of crown rot can be predicted with reasonable accuracy from crown rot incidence and yield (a surrogate for biomass) of the preceding crop (1). The equation is:

\[ X_1 = I \sqrt{(X_0 Y_0)} \]

where \( X \) is incidence, \( Y \) is yield and \( I \) is an infection constant which typically ranges from 4.5-6. The constant appears to vary with location rather than with variety.

Rotations and fallows If wheat is not sown in the autumn following harvest, crown rot inoculum declines exponentially (2). This can be represented as:

\[ \sqrt{(X_t Y_t)} = \sqrt{(X_0 Y_0)} e^{-dt} \]

where \( d \) is a decomposition constant, and \( t \) is the number of years out of wheat. Decomposition is actually related to thermal time corrected for rainfall (2) but can be approximated quite well by using an annual constant, which is typically about 0.4.

Convergence over time If the two equations above are used to model crown rot incidence over a series of cropping cycles, incidence converges to an equilibrium value irrespective of the initial incidence (Figure 1). The equilibrium can be calculated as:

\[ X = I^2 Y (1-d)^2 \]

Examples In continuous wheat, assuming \( I = 5.5 \) and yield is 2 t/ha, crown rot will reach an equilibrium of approximately 30 times yield, or 60% (Figure 1). This reflects what was seen in the field data from which the first equation was derived (1). In a 2-year rotation, such as wheat-chickpea, with a decomposition coefficient of 0.4 per year, the equilibrium incidence will be approximately 9 times yield. For a yield of 2.5 t/ha this would give crown rot incidence of 25-30% (Figure 1), which is similar to that reported in several studies. For a 3-year rotation such as wheat-sorghum, equilibrium incidence will be approximately 4 times yield, or 15% for a yield of 4 t/ha. This is similar to the incidence reported after three rotation cycles in the field (3).

DISCUSSION
The model uses a simplified mathematical description of increase in crown rot incidence between seasons, and decline in inoculum during rotations. It is not intended to give an accurate prediction of crown rot incidence under all conditions, but rather to allow generalisations about how the disease behaves. These can then be used in extension or as a way of understanding how management and environment affect the disease.

In this model, the incidence of disease can be altered by manipulating the infection constant \( I \), decomposition rate of residues, or time between wheat crops. Time between crops has by far the largest effect, highlighting the importance of rotation in management. Precision sowing and resistance will affect the infection constant \( I \). Environmental effects on residue decomposition can be included (2). For example, the decomposition constant declines from 0.4 to 0.3 at the western edge of the grain belt, leading to a doubling of crown rot incidence in a 3-year rotation.

The model only describes incidence. In any season, losses will also be determined by disease severity. Environmental factors such as temperature and rainfall affect severity. Management factors including nutrition, sowing rate and genetic resistance and tolerance act most directly on severity. Appreciating the place of each of these factors in epidemiology of the disease may help to manage it more effectively.

REFERENCES

Figure 1. Modelled disease progress of crown rot in continuous wheat with a yield of 2 t/ha, 2-year rotation with a yield of 2.5 t/ha, and 3-year rotation with a yield of 4 t/ha.
INTRODUCTION
Crown rot of wheat, caused by *Fusarium pseudograminearum*, is a typical monocyclic disease in which epidemics progress over multiple years. The pathogen survives in infected residue that serves as the source of inoculum in succeeding crops (1). Therefore, the location and number of infected plants influences the long term behaviour of epidemics. Spatio-temporal analyses of plant disease epidemics usually deal with the spread of polycyclic diseases within individual seasons, and are not readily applicable to diseases like crown rot.

This paper describes the development of a model for the spread of crown rot within a field over successive years, based on assumptions about the mechanism of infection and dispersal. The aim was to produce a tool which could be used to predict the spatial effects of management, such as row spacing and orientation.

MATERIALS AND METHODS
The model assumed that in each year, wheat was sown in rows between the rows of residue from the previous year. Inoculum from the residue of each infected plant therefore came into contact with a specified number of plants in the rows on either side. The probability that each of these plants would become infected was also specified (Figure 1).

![Figure 1](image)

Figure 1. Simulation model for spread of crown rot. A) In the first year, infected plants are randomly allocated to positions in the 'field'. B) In the next year, inoculum from each infected plant is assumed to come into contact with a specified number of adjacent plants (here 5). C) Each plant in contact with inoculum has a specified probability (here 0.6) of becoming infected. The success of infection is determined individually for each plant using a random number generator.

Simulation modelling was done in a spreadsheet with a Monte Carlo simulator add-in. A population of 1000 plants was randomly seeded with 40 infected plants, and simulations run for 10 years with different combinations of number of plants contacted and of probability of infection. Spatial aggregation was tested at intermediate incidence (40-45%) using SADIEShell (2). Parameters of the epidemics were compared with previously published field data from a series of trials at Moree, NSW (1).

RESULTS
The published field data showed that as crown rot incidence increased, the number of plants infected from the residue of each plant decreased (Figure 2). The relationship was best described by a power function nonlinear regression. A function derived from a simulation where the residue of each infected plant contacted 12 plants in the next season with a probability of infection of 0.66 gave a very close fit to the field data ($R^2 = 0.96$, $P < 0.001$; Figure 2). The simulation gave a sigmoïdal curve for disease incidence over time, with maximum incidence equal to the probability of infection (not shown), which matched previous field observations.

Spatial analysis of three repeats of the simulation using SADIE gave $I_o > 3$ and $P_s < 0.001$ indicating highly significant aggregation of diseased plants.

![Figure 2](image)

Figure 2. Number of plants infected in the next season by each crown rot-infected plant relative to incidence in the field (open circles) and simulated (closed circles). Simulation assumed 12 plants were contacted by each infected plant, with probability of infection of 0.66. Line of best fit to simulation shown.

DISCUSSION
Epidemiological characteristics of field epidemics of crown rot, including disease progress, maximum incidence and aggregation could be described using a relatively simple mechanistic model with just two parameters. The model is likely to be over-simplified: for example, the probability of infection would be expected to be higher for plants closer to the crown of infected residues than further away. The model also did not distinguish between infection directly from the residue and secondary spread from infected plants. However, it does provide a simple method for generating hypotheses about spatial aspects of the disease. In the field data used, 12 plants contacted was equivalent to a radius of 17 cm around the point where each diseased plant in the previous season grew. The effect of location of residue relative to the newly-sown rows of plants can therefore be predicted, allowing prediction of the effects of row spacing and orientation, and precision planting, on disease progress.

REFERENCES
GENETIC DIVERSITY OF *PLASMODIOPHORA BRASSICAE* IN AUSTRALIA

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INTRODUCTION

Clubroot of cruciferous crops, caused by the soil-borne obligate biotroph, *Plasmodiophora brassicae* Woronin, is an important disease of *Brassica* crops worldwide. *P. brassicae* colonizes the roots of host plants which become swollen and form galls. The infection interferes with the plant’s water and nutrient transport. Effective/practical control is difficult.

Collections of the pathogen vary in virulence and also vary genetically (1, 2) but few of those tested previously were from Australia. An understanding of the diversity in pathogen populations is important for the development of cultivars with effective and durable resistance to this disease (1). This study investigates the genetic variation among different collections of *P. brassicae* in Australia using DNA markers.

MATERIALS AND METHODS

Genomic DNA of *P. brassicae* was extracted from spores contained in root galls. DNA from 10 collections of *P. brassicae* from farms in Australia was screened for polymorphisms using RAPD and microsatellite primers (1). This was compared to DNA from a single spore isolate sourced from Germany (isolate 11). PCR amplification was conducted using 55 RAPD primers and 4 microsatellite primers.

RESULTS AND DISCUSSION

RAPD and microsatellite analysis indicated that the population of *P. brassicae* in Australia is diverse. All 55 RAPD primers produced clear bands ranging in size from 180 to 3500 base pairs and showed a high level of polymorphism. Microsatellite primers also produced clear banding patterns with bands ranging from 200 to 3200 base pairs. The high level of polymorphism observed amongst Australian collections (Figures 1 and 2) indicates genetic variation within the population. This may correlate with virulence (1,2).

An example of the banding patterns obtained using primers OPB7 and HKB17/9 are shown in Figures 1 and 2. Some of the primers amplified similar-sized bands in a number of collections, but the presence of additional bands and the resulting distinct banding patterns of some collections indicate genetic differences between them. Some collections appeared to have unique multilocus molecular genotypes. In particular, collections 3, 6 and 10 and single spore isolate 11 were genetically different from the other collections.

Previous research indicates that *P. brassicae* populations are relatively homogenous (3). However, the current molecular analysis suggests a greater degree of heterogeneity. The differences observed in diversity within populations may reflect variation related to regions (eg. collection 3 was from Devon Meadows VIC, 6 was from Cora Lynn VIC; 10 was from Manjimup WA and isolate 11 was ‘e3’, a German single spore isolate).

RAPD and microsatellite primers (1, 2) proved a useful means of investigating polymorphism in Australian collections of *P. brassicae*. In particular, RAPD primers OPA 1, 8, 11; OPB 3, 7, 20; OPM 2, 13, 16; and microsatellite primers (GACA)\(^4\), HKB 17/9, HKB 23/52 revealed key differences between the collections of *P. brassicae* investigated in this study.

![Figure 1](Image)

**Figure 1.** The variation between different *P. brassicae* collections tested with RAPD primer OPB7 (Lane M, DNA ladder, Lanes 1-10, collections of *P. brassicae* from different regions in Victoria, Australia, Lane 11, German single spore isolate ‘e3’).

![Figure 2](Image)

**Figure 2.** The variation between different *P. brassicae* collections tested with microsatellite primer HKB17/9 (Lane M, DNA ladder, Lanes 1-10 collections of *P. brassicae* from different regions in Victoria, Australia; Lane 11, German single spore isolate ‘e3’).

The high degree of polymorphism observed may be used to type collections and may allow the development of a molecular assay for virulence among strains to replace the laborious and slow differential host assay (The European Clubroot Differentials).

The potential existence of mixed pathotypes of *P. brassicae* within a single root gall remains a complicating factor. We are currently investigating this possibility using the techniques described here to study changes in the pathogen population through multiple host generations.

ACKNOWLEDGEMENTS

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EFFECT OF THE BIOPESTICIDE BACILLUS THURINGIENSIS ON POPULATIONS OF NON-TARGET NEMATODES

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INTRODUCTION
Bacillus thuringiensis (Bt) is a widely used bioinsecticide with a greater than 50% share of the microbial control market. The product Foray® 48B, which contains formulated Bt, is used for control of caterpillars in situations such as ground spraying orchards and in aerial applications. In both cases it can come into contact with soil by run-off or overspray (1). As such Bt is likely to be encountered by non-target organisms, including soil nematodes.

There are previous reports of Bt having deleterious effects on nematodes (2), but these are often experiments conducted in soil-free media. In order to see if any non-target effects on nematodes occur in soil, a pot experiment was conducted with a range of Bt rates applied to soil.

MATERIALS AND METHODS
Soil (Templeton silt loam, to 5 cm depth) was taken from beneath pasture near Lincoln in Canterbury, New Zealand in January 2003. Soil was hand crumbled and placed into 32 pots which were then sown with three seeds each of Yatsyn perennial ryegrass (Lolium perenne) and Grasslands Sustain white clover (Trifolium repens). Pots were maintained in a controlled environment room with overhead watering for eight weeks. Four rates of B. thuringiensis subsp kurstaki (from Foray® 48B) were established in eight replicate 15 cm diameter pots per rate by adding 10 ml of distilled water (control); the recommended field rate (5L/ha); 100× and 1000× the field rate. Pots were then maintained as before. Four pots for each rate were sampled 1 and 2 weeks after B. thuringiensis inoculation. Shoots were removed to ground level, but roots were retained so they were included in the extraction process. Nematodes were extracted from 200 g of soil per pot using the tray method described by Bell and Watson (3). Total nematodes were counted using a Doncaster dish then fixed in formalin. Approximately 100 nematodes per sample were discriminated to feeding group (4), or genera in the case of bacterial-feeding and plant-parasitic nematodes. Data were analysed by ANOVA (with ln(n+1) transformation).

RESULTS AND DISCUSSION
There was a significant difference in total nematode and bacterial feeding nematode abundance only for the 1000× rate and this was apparent from 1 week after Bt soil inoculation (Figure 1). Bacterial-feeding Rhabditidae nematodes were largely responsible for the increase in total bacterial nematode abundance, being 3.5 and 19.2 times more abundant (P<0.05) in the 1000× treatment than in the untreated at weeks 1 and 2 respectively (data not shown). Pristionchidae bacterial feeders significantly increased in response to the 1000× compared to both the 1× and 100× treatments at Week 2 (P<0.05). For the 1× and 100× rates there was no consistent or significant difference in the abundance of any of the bacterial feeding groups, compared to the control pots.

Other than the bacterial-feeding nematodes there was no significant difference in abundance of any of the feeding groups or genera of nematodes identified. Pratylenchus were the predominant plant-parasitic nematodes in the soil at both harvests with only small populations of Heteroderus nematodes observed.

In environmental terms, the addition of the 1000x rate of Bt represents a large pulse (ca. 1 × 10 8/ g soil) to the resident bacterial flora (ca. 1 × 10 9/ g soil), and indicates the reason for the large response by the bacterial feeding nematodes. Reaction to bacterial addition was also rapid with increases observed one to two weeks after inoculation. There appeared to be no consistent effect of addition of Bt to soil on other nematode feeding groups.

It appears that soil application of the field rate of Foray had little measurable effect on the nematode fauna within the 2 week period of this study. At very high rates it appears bacterial-feeding nematodes use the Bt as a food source, possibly reducing the persistence of excess bacteria in the soil. Bt spores have been observed to decline by ca. 90% in 2 weeks in field soil (1) and it is possible this is due, at least in part, to nematode feeding.

ACKNOWLEDGEMENT
This study was funded by FRST Contract C10X0601.

REFERENCES
INTRODUCTION
Rhizoctonia solani and Sclerotinia sclerotiorum are important soilborne fungal pathogens of potato and brassicas, respectively. Biocontrol agents are available for these pathogens overseas, but are not registered in New Zealand because these organisms are considered exotic and of potential environmental risk. It would therefore be advantageous to isolate and develop local organisms for biocontrol of these pathogens.

In this study, bacteria were isolated from potato and brassica plants and soils from three geographical regions in New Zealand: Auckland, Manawatu, and Canterbury. This paper concentrates particularly on the diversity of bacteria isolated from the Canterbury region.

MATERIALS AND METHODS
Isolation of bacteria Roots, leaves, stems and tubers of potato plants, and roots, leaves, stems, and florets of bokchoy, cauliflower, and broccoli plants were sampled from two locations in Canterbury, a home garden and commercial vegetable production farm. For each plant, two samples were taken from each tissue type. Samples were washed for 1 min either in sterile reverse osmosis (RO) water or 80% ethanol. Both treatments had two further rinses in sterile RO water. Tissue samples were individually macerated in drops of sterile water, streaked onto nutrient agar plates, which were then incubated at 25°C until discreet colonies formed. Single colonies were selected, subcultured at least twice, then stored in 25% glycerol at -80°C.

16S rRNA analysis Bacteria were cultured from storage onto nutrient agar. DNA was extracted and amplified using the REDExtract-N-AmpTM Plant PCR kit (Sigma). The variable portion of the 16S rRNA gene was targeted with primers (F27 5’ AGAGTTTGATCCTGGCTCAG 3’, R1494 5’ CTACGGTTACCTTGTTACGAC 3’). PCR parameters were: 94°C for 3 min, 30 cycles of 94°C 1 min, 57°C 1 min, 72°C 2 min, followed by 10 min at 72°C. PCR products were analysed by agarose gel electrophoresis, and purified using an Agencourt® AMPure XP kit (Beckman Coulter).

Sequencing and Analysis Sequencing was carried out using ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kits and PCR cleanup was with Agencourt® CleanSEQ Dye-Terminator Removal (Beckman Coulter). An ABI Prism 3130xl Genetic Analyzer was used. Sequences were analysed using Sequencher 4.9 software and identified with EzTaxon2.1.

RESULTS
A total of 608 bacterial isolates were collected from sites around New Zealand, and 473 (79%) have been identified to genus by sequence analysis. In the Canterbury region, a comparison of 144 isolates from potato and 162 isolates from brassicas were diverse, representing a total of 37 genera. Of these, 13 genera were isolated at a frequency greater than 2 percent (Table 1). Pseudomonas was the most prevalent genus identified from brassica plants, with both Pseudomonas and Bacillus isolated at greater than 10 percent frequency from potato plants. Pseudomonas and Bacillus were isolated from almost all tissues of potato and brassicas sampled, and were found regardless of surface sterilisation. The genera Microbacterium, Variovorax and Flavobacterium were frequently isolated from both plant species. Serratia was isolated from a number of brassica samples, but not from potato.

Table 1. Numbers of isolates of the most frequently isolated bacterial genera from either potato or Brassica plants.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Potato</th>
<th>Brassica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthrobacter</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Bacillus</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Candidatus</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Microbacterium</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>25</td>
<td>76</td>
</tr>
<tr>
<td>Psychrobacter</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Rhizobium</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Rhodococcus</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Serratia</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Sphingobacterium</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Stenotrophomonas</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Variovorax</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

DISCUSSION
Bacillus and Pseudomonas are commonly occurring bacterial genera of which several species have been used with success in in vivo experiments as biocontrol agents against R. solani and S. sclerotiorum. S. sclerotiorum has also been shown to have biocontrol properties (1).

Preliminary in vitro and in vivo screenings against R. solani and S. sclerotiorum have shown some isolates to have potential as biocontrol agents against these pathogens. The most promising isolates with be further evaluated to determine their biological attributes and biological control mode of action.

ACKNOWLEDGEMENTS
This work was funded by the New Zealand Foundation for Research, Science and Technology. Norma Merrick sequenced bacterial isolates. L Loguercio was funded by CAPES and UESC (Brazil).

REFERENCES
BIOLOGICAL CONTROL OF RHIZOCTONIA SOLANI IN PERENNIAL RYEGRASS USING TRICHODERMA ATROVIRIDE ISOLATES

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Prashant.Chohan@lincolnuni.ac.nz

INTRODUCTION
In a previous study, a cohort of Trichoderma atroviride isolates with biocontrol and plant growth promoting attributes for pasture species was identified. Disease suppression was obtained against damping-off and root rot pathogens including Rhizoctonia solani. For greater field efficacy, Naseby et al., (1) suggested integrating biocontrol and plant growth promoting strains rather than single action inocula. In some investigations (2), using a mixture of Trichoderma isolates has yielded better results than those for individual isolates. We, therefore, investigated the effects of four individual T. atroviride isolates and their mixture on seedling emergence and subsequent plant growth of perennial ryegrass challenged with R. solani.

MATERIALS AND METHODS
A glasshouse pot experiment was conducted during summer 2009 (February-April) using a randomised block design (4 blocks with 5 replications of each treatment/block). Mean temperature in the glasshouse was 20±2°C during the experimental period. T. atroviride isolates were grown on sterile wheat bran and peat mixture to yield 10^6 colony forming units (CFU)/g and were tested for their ability to suppress disease in perennial ryegrass (Lolium perenne var Bealey) when challenged by artificially inoculated R. solani. The potting-mix (PM) was first inoculated with R. solani (0.5% w/w mycelial inoculum multiplied on wheat bran) followed by individual T. atroviride isolates and the mixture (with equal proportions of the four isolates). The Trichoderma treatments were applied @ 2g/pot to give 10^6 CFU/g of potting-mix. 50 seeds/pot were sown and the pots were watered regularly to avoid water-stress. Seedling emergence was counted 15 days after sowing. Shoot and root dry matter (DM) was determined at 9 weeks after sowing.

RESULTS
Seedling emergence: Emergence from the PM control was 88% (44/50) but the presence of the pathogen reduced this to 32% (16/50). All the Trichoderma treatments significantly increased emergence over the pathogen control, but all had a lower emergence than the PM control. Emergence also differed among the Trichoderma treatments, with LU132 and LU584 producing greater emergence than LU140, LU633 and the mixture (Table 1). Root DM for three of the T. atroviride treatments was greater than the pathogen control with LU132 equal to the PM control (Table 1). When expressed as DM/plant, root DM did not differ among the treatments and controls. Shoot DM for all Trichoderma treatments was significantly greater than the PM control (data not presented).

Table 1. Effect of four Trichoderma atroviride isolates (applied individually and as a mixture) on perennial ryegrass seedling emergence and shoot and root dry matter when grown in potting-mix inoculated with Rhizoctonia solani.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of seedlings emerged</th>
<th>Dry matter (g/pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>PM control</td>
<td>44 a</td>
<td>2.8 a</td>
</tr>
<tr>
<td>Pathogen control</td>
<td>16 c</td>
<td>2.0 c</td>
</tr>
<tr>
<td>LU132</td>
<td>31 b</td>
<td>2.8 a</td>
</tr>
<tr>
<td>LU140</td>
<td>25 d</td>
<td>2.4 b</td>
</tr>
<tr>
<td>LU584</td>
<td>29 bc</td>
<td>2.6 ab</td>
</tr>
<tr>
<td>LU633</td>
<td>27 cd</td>
<td>2.6 ab</td>
</tr>
<tr>
<td>Mixture</td>
<td>25 d</td>
<td>2.5 b</td>
</tr>
<tr>
<td>LSD (P≤0.05)</td>
<td>3.9</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Note: Values followed by different letters in each column are significantly different.

DISCUSSION
In the presence of the pathogen, all Trichoderma treatments increased seedling emergence, ranging from 56% to 94%. LU132 and LU584 gave the best emergence results and this translated into greater shoot and root dry weights per pot. However, on an individual plant basis, there was no difference in dry weight measurements between any of the Trichoderma treatments and the pathogen control. This can be explained by the growth compensation observed when plant populations were low.

In previous field experiments, the four isolates combined in a mixture prototype product produced significant seedling establishment and yield benefits (D.R.W. Kandula, unpublished data), although any pathogen influence was not always known. In this study, all four isolates were able to reduce the effects of R. solani on seedling emergence with LU132, performing the best. However in the field environment, the mixture is likely to have the greatest commercial potential since it combines the biocontrol and growth promotion properties of four isolates and, therefore, is likely to provide a wider spectrum of activity with more general yield benefits across multiple sites. Future research will focus on gaining a greater understanding of the synergistic and/or inhibitory interactions amongst the Trichoderma isolates.

REFERENCES
INTRODUCTION
Gaeumannomyces graminis var. tritici (Ggt) is a soil-borne pathogen that causes the disease take-all in cereals. Manganese (Mn) in both soil and seed is important for cereal defences against Ggt infection (1). The wheat growing region of Canterbury has some Mn deficient soils in which foliar application of MnSO4 has improved grain yields while excessive lime application exacerbated Mn deficiency (2). The aim of this work was to investigate the effect of seed and soil Mn concentrations for information on Ggt buildup and take-all management.

MATERIALS AND METHODS

Seed and soil Mn Wheat cv. ‘Torlesse’ seed was sourced from eight sites, four of which were low in Mn. Soil samples were analysed for available Mn (0.05 M EDTA extraction).

Seed Mn and take-all 10 wheat seed-lines (each of a different cultivar) of known Mn content were sown as a second wheat in a low Mn site (58 mg Mn/kg, pH 6.0) in a replicated (n = 4) trial. Pre-sowing inoculum of Ggt at the site, analysed by the PreDicta B diagnostic service (3), was in the high risk category at 271 pg Ggt DNA g/soil. Root systems were assessed for incidence and severity (TAI) of take-all lesions at early grain fill and the percentage area of take-all patches at early senescence.

Ggt and soil Mn Soil was collected (10 cm-deep, ~30 kg from 50*50 m area) from six commercial fields (A,B,C,D,E,P) 1–2 months after the harvest of a first wheat crop that followed a break crop. Sites P and E were the wheat fields after harvest and take-all was assessed in the following second wheat crop. Sites A, B, and C were from the previous first wheat. Sites D and E were from only six sites. Take-all incidence and severity in a second wheat at a low soil Mn site were high but also reflected the high take-all risk at the site. The study of more low Mn sites is required to better understand the effects of soil Mn concentrations on take-all and the build-up of inoculum.

RESULTS
Seed and soil Mn Mn content in ‘Torlesse’ seed had a similar lower range between three low (22-30 mg/kg) and four non-deficient sites (25-51 mg/kg). Seed from a trial area that had been limed and had no MnSO4 applied had very low Mn concentrations (8 mg/kg).

Seed Mn and take-all Take-all was severe (TAI range of 34-65) in the field trial, but the seed Mn content (19-35 mg/kg) was not correlated with take-all severity, incidence or the area of take-all patches across 10 cultivars.

Ggt and soil Mn Soil Mn concentrations in the low areas were, on average, a third those in higher areas (Table 1). Ggt levels were not higher from low Mn areas, although Ggt levels were very high (5806 pg Ggt DNA g/soil) for soil collected from take-all patches at site P.

Soil Mn and take-all Take-all incidence and severity in second wheat crops were correlated with pre-sowing Ggt levels following the previous first wheat. The low Mn site had a relatively high pre-sowing Ggt level (271 pg DNA/g soil) and 68% of plants infected with moderate severity at early grain fill (TAI of 26.2). Early senescence was also correlated with take-all and with presowing Ggt. The low Mn site had the fourth highest incidence of early senescence of the 18 Ggt tested second wheats.

DISCUSSION
Seed Mn content differs between cultivars (1). A small survey of ‘Torlesse’ seed produced at eight sites, including low Mn areas, indicated that seed Mn concentrations where MnSO4 was applied were above the Mn seed deficiency point of 11.5 mg Mn/kg (4). In a field trial using seed-lines of different cultivars, take-all infection at early grain fill and area of take-all patches at early senescence were not influenced by seed Mn concentrations. However, seed with very low Mn content was not evaluated. Seed Mn may be more important at sites with low inoculum. In a survey of 18 wheat fields, low soil Mn did not appear to facilitate greater Ggt build-up in first wheats, but results are limited, being from only six sites. Take-all incidence and severity in a second wheat at a low soil Mn site were high but also reflected the high take-all risk at the site. The study of more low Mn sites is required to better understand the effects of soil Mn concentrations on take-all and the build-up of inoculum.

ACKNOWLEDGEMENTS
PGG-Wrightsons Ltd for the second wheat cultivar trial.

REFERENCES

SOIL AND SEED Mn EFFECTS ON TAKE-ALL
S L BithellAC, D CurtinA, A McKayB, M G CromeyA

6th Australasian Soilborne Diseases Symposium, 2010

Table 1. Soil Mn, pH and Ggt levels for three (a) non-deficient (b) and three low Mn sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Mn mg/kg</th>
<th>pH</th>
<th>Ggt DNA g/soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>159</td>
<td>6.0</td>
<td>47</td>
</tr>
<tr>
<td>B</td>
<td>162</td>
<td>6.0</td>
<td>48</td>
</tr>
<tr>
<td>P</td>
<td>106</td>
<td>5.8</td>
<td>1675</td>
</tr>
<tr>
<td>mean</td>
<td>142</td>
<td>5.9</td>
<td>590</td>
</tr>
<tr>
<td>C</td>
<td>57</td>
<td>5.9</td>
<td>81</td>
</tr>
<tr>
<td>D</td>
<td>39</td>
<td>5.6</td>
<td>6</td>
</tr>
<tr>
<td>E</td>
<td>39</td>
<td>6.1</td>
<td>55</td>
</tr>
<tr>
<td>mean</td>
<td>41</td>
<td>5.9</td>
<td>36</td>
</tr>
</tbody>
</table>
INTRODUCTION
Gaermannomyces graminis var. tritici (Ggt) is a soil-borne pathogen that causes the disease take-all in cereals and grasses. Soil factors such as pH may influence infection, as a result of indirect effects on availability of elements such as manganese (1, 2). Non-Mn deficient field soils within the pH range for Canterbury wheat fields were used to study pH effects on take-all infection.

MATERIALS AND METHODS
Soil was collected in 2008 from commercial fields (0–10 cm depth, ~30 kg from 50*50 m area) 1–2 months after the harvest of a wheat crop (fields A, B and P). Wheat following a break crop), and from a site that had been in ryegrass since 1999 (M). Soils were dried to ~3% moisture and Ggt inoculum (Ggt ratio to DW 1, 2). Non-Mn deficient field soils within the pH range for Canterbury wheat fields were used to study pH effects on take-all infection.

RESULTS
Table 1. Percentage seminal root (95% confidence limits) infection for uninoculated (a) and inoculated (b) soil B.

<table>
<thead>
<tr>
<th>pH</th>
<th>5.0</th>
<th>5.6</th>
<th>5.9</th>
<th>6.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>30.4</td>
<td>42.0</td>
<td>43.7</td>
<td>58.9</td>
</tr>
<tr>
<td>b</td>
<td>30.0–65.7</td>
<td>48.4–82.1</td>
<td>48.2–81.6</td>
<td>35.9–71.7</td>
</tr>
</tbody>
</table>

DISCUSSION
Increases in take-all infection were observed with increasing pH, but differences were not always significant. Overall, infection was lower in the second pot trial than the first. This was probably due to the result of overheating in the glasshouse in a 3-day period after establishment. The area of runner hyphae indicated that lesion area could have increased further under suitable conditions. Significant pH x soil interactions may indicate other factors may be involved. It would be useful to evaluate the effects of pH on Mn deficient soils.
SEED POTATO CERTIFICATION: ITS VALUE TO INDUSTRY

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INTRODUCTION
Certified seed potatoes underpin the multi-million dollar national potato industry, including the increasing export markets. Total value of annual potato production in Australia is around $470 million (Australian Bureau of Statistics). In 2005–06, Australia exported 52,000 tonnes of potatoes or potato products, or about 4% of annual production, at a value of $39m.

All sectors (fresh/table and processing) of the Australian industry rely upon certified seed production. An effective seed certification scheme ensures the efficient production of a stable food product to consumers.

The Victorian seed potato certification scheme has made significant achievement and contributions to the productivity and growth of the Australian potato industry over the past 70 years.

The successful development and administration of the seed certification scheme in Victoria has meant that there has been:
- Reliable high health seed production that meets national seed standards.
- Increased yield and product quality of commercial crops in the fresh and processing industries.
- Enhanced efficiency in the use of natural resources, including land and water.
- Management of tuber borne diseases, including many viral diseases that severely limit yield and quality.
- Reduced reliance on pesticides to manage pest problems and a high adoption of integrated pest management practices.

Seed potato certification contributes to the increased production of potatoes in Australia despite declining areas of production. Although the area of land under potatoes has declined about 26% in the last 100 years, potato production has risen five-fold (1). In fact, the potato yield per hectare has continued to increase with the adoption of modern farming practices including the implementation of seed potato certification schemes around 1937. It is therefore reasonable to assume that certified seed potatoes will continue to lend stability to a crop that shares a significant part of our diet and economy.

OBJECTIVE OF SEED POTATO CERTIFICATION
Seed potato certification programs are designed and administered as a means to provide reasonable assurances of seed quality.

The reference to seed is not true botanical seed. It is a reference to potato tubers which serve as vegetative units for propagation of plants which will produce the new potato crop (2). Many potato diseases are systemic in potato plants and can be carried in or on the surface of such ‘seed’ tubers.

Monitoring of seed crops for disease is largely by visual inspection supported by laboratory testing using ELISA or PCR technology. The following diseases are monitored in the Victorian seed potato scheme:
- Blackleg and related soft rots caused by Erwinia spp.
- Bacterial wilt, caused by Ralstonia solanacearum
- Ring rot, caused by Clavibacter michiganense pv. sepedonicum
- Powdery scab, caused by Spongospora subterranea
- Black scurf, caused by Rhizoctonia solani
- Silver scurf, caused by Helminthosporium solani
- Gangrene, caused by Phoma exigua
- Wilt, dry rot, caused by Fusarium spp.
- Wilt, caused by Verticillium spp.
- Black dot, caused by Colletotrichum cucodes
- Late blight, caused by Phytophthora infestans
- Common scab caused by Streptomyces spp.
- Potato leafroll virus (PLRV), potato virus A (PVA), potato virus M (PVM), potato virus X (PVX), potato virus Y (PVY), tomato spotted wilt virus (TSW), and potato spindle tuber viroid (PSTV)
- Calico, caused by Alfalfa Mosaic Virus

The tolerances of these diseases for seed certification vary from zero tolerance to an acceptable rating determined by incidence and severity.

ROLE IN BIOSECURITY
The Victorian seed potato certification scheme has a considerable role in the biosecurity of the National Potato Industry for exotic pests such as Potato Cyst Nematode (PCN). The ViCSPA operated scheme is the only seed scheme in Australia that has a long history of soil testing for PCN, thereby minimising the spread of the PCN throughout Australia and helping to determine pest free areas.

With trained field certification officers frequently monitoring around 2000ha of seed crops, there is the ability for early detection of new incursions of pests and diseases.

CONCLUSIONS
Seed potatoes are among the least expensive of inputs, but are the most important contribution to yield and quality of a commercial potato crop (2). As gross margins of potato crops continue to decrease, the value of seed certification will be further enhanced.

Seed potato certification is of extreme value to industry in terms of minimising the risk of disease and enhancing potential yield and quality.

ACKNOWLEDGEMENTS
ViCSPA is a non for profit industry organisation that is independently operated.

REFERENCES
EVALUATION OF THE EFFICACY OF AVICTA AS SEED TREATMENT ALONE OR IN COMBINATION WITH FUSARIUM OXYSPORUM STRAIN 162 FOR MANAGEMENT OF ROOT-KNOT NEMATODE ON TOMATO

A. A. Dababat, A. C. Watrin, A. Cochran, M. Klix and R. A. Sikora

INTRODUCTION
Avicta (active Abamectin) as a seed treatment is used for the control of plant parasitic nematodes especially root-knot nematodes in the field since only low amounts of active ingredient are required to achieve adequate protection in the most sensitive stages of tomato root growth and development (1, 2). The non-pathogenic endophytic Fusarium oxysporum strain 162 (FO162) has been selected for its potential to limit damage by root-knot nematode on different vegetable crops. The aim of this study was to determine the potential synergistic effects of ‘bio-chem’ seed treatments containing nematicidal and/or nematostatic agents in different combinations on the control of root-knot nematode in tomato. A nematicidal biopesticide, the mutualistic endophytic antagonist (Fusarium oxysporum 162) was used for long term control and the nematicide Avicta (Abamectin) for mid-term protection toward the sedentary parasite in the roots of tomato.

MATERIALS AND METHODS
Untreated tomato seeds or seeds treated with 0.3 mg Abamectin/seed were planted in multiple planting trays containing a field soil: sand mixture (v:v, 1:1) plus 10% seedling substrate (v:v), hereafter referred to as soil. One-week-old tomato seedlings were inoculated with a spore suspension containing 1x10^5 colony forming units of FO162.

A mixture of 1000 nematode eggs and second stage juveniles per 100 cm^3 soil was incorporated into the soil in 800 cm^3 pots. Pots were then covered with plastic wrap to maintain humidity and transferred to a greenhouse bench where they were incubated for 10 days. During this time sufficient moisture levels were maintained to ensure the viability of the nematode inoculum.

Tomato seedlings of the different treatments were transferred to the nematode treated soils 10 days after FO162 inoculation. Pots were placed in a greenhouse at 22 ± 2°C with a 16 h light period and watered daily. Eight weeks after transplanting, nematode damage and plant growth parameters were recorded.

RESULTS AND DISCUSSION
Seedlings treated with Avicta or FO162 resulted in a significant (P ≤ 0.05) reduction in nematode gall index when compared to the control. However, Avicta showed higher efficacy in reducing the number of galls compared to FO162. However, there was no significant difference between treatments. Combining the two control agents gave slightly higher reduction in nematode gall index despite the fact that there was no significant difference at (P ≤ 0.05) for both Avicta and FO162 when applied alone (Figure 1).

The effect on egg mass production, which is a measure of nematode development over time, was significantly reduced when the seedlings were treated with Avicta or FO162. The reduction in the number of egg masses was probably caused by a delay in penetration of the roots due to repellent activity of FO162 on the juveniles. In previous studies FO162 affected nematode penetration into tomato roots when applied at seeding stages (3, 4).

REFERENCES
INTRODUCTION
Phytophthora root rot, caused by *Phytophthora cinnamomi*, is ubiquitous throughout avocado producing regions of the world and is considered the most important soilborne disease. Other soilborne diseases are considered of minor importance to the Australian industry, but may contribute to significant productivity losses on individual orchards, or within regions. Those of recent concern to the local industry include brown root rot caused by *Phellinus noxius* (1), Verticillium wilt, caused by *Verticillium dahliae* (2). Phytophthora trunk canker, caused by *P. cinnamomi* (3), and death of young trees with associated isolations of *Cylindrocarpon destructans*, *Cylindrocladiella parva* and/or *Cylindrocladium parasiticum*.

Phytophthora root rot and research on its management is being covered in a separate poster at this meeting.

MATERIALS AND METHODS
 **Brown root rot** Several properties were visited in the Atherton Tablelands and Bundaberg/Childers (QLD) and northern NSW production areas. Most of these were suspected of having brown root rot. Dead trees and those in the immediate vicinity were examined and samples from viable infection stockings taken for isolation and identification to confirm presence of *P. noxius*.

**Verticillium wilt** In spring 2009 foliar symptoms of sudden wilt followed by browning and apparent branch death were noted in an orchard at Bundaberg where tree thinning or severe pruning occurred in winter. Affected branches were inspected and isolations made for confirmation of the likely causal organism.

**Phytophthora trunk canker** Weeping cankers usually restricted to the lower trunk are observed infrequently in our trials, usually only in a small number of trees. Release of white coloured perseitol, the 7-carbon avocado sugar alcohol, often occurs (Plate 1). In 2009 the incidence and severity of trunk canker in a rootstock trial near Childers was recorded.

RESULTS AND DISCUSSION
**Brown root rot** *P. noxius* was confirmed present on 17 properties visited on the Atherton Tablelands (also confirmed in mango at 2 of these orchards), 3 in Bundaberg/Childers area (and suspected on another 2 in the region), one orchard at Maleny in the Sunshine Coast hinterland and 2 orchards in northern NSW. The most common symptoms observed were rapid tree wilting, decline and death, progressive death of trees along a row (caused by root to root infection), an infection ‘stocking’ at the base of the trunk (an encrusted mass of soil, twigs etc. held together by brown mycelium sometimes with a white margin, which melanises with time). Dead trees did not always have an obvious infection stocking on the trunk, and conversely, large trees with conspicuous infection stockings were often not dead.

**Verticillium wilt** Examination revealed brown/grey streaking of the vascular tissue, and *V. dahliae* was confirmed in cultures of isolated material. It was thought that major limb removal during the winter may have stressed or injured the feeder roots, which, combined with the cooler wet weather, favoured *V. dahliae* infection of roots and subsequent colonisation of vascular tissue in spring.

**Phytophthora trunk canker** Around 13% of trees in the trial had trunk canker, being significantly more severe in one Mexican race rootstock compared with 3 other Guatemalan, 1 West Indian and 1 other Mexican race rootstocks. *P. cinnamomi* was isolated from a representative sample of trunk cankers. Trunk canker is of minor importance in Australia and management of Phytophthora root rot will reduce the impact of trunk canker in most cases.

REFERENCES
CHARACTERISATION OF RHIZOCTONIA SOLANI ANASTOMOSIS GROUP 2-1 FROM POTATO TUBERS IN NEW ZEALAND

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INTRODUCTION

Rhizoctonia solani (teleomorph, Thanatephorous cucumeris) is one of the most economically important pathogens of potato. This fungus causes significant economic losses due to yield depression and reduced tuber quality. The most common symptoms associated with this pathogen are stem canker and tuber black scurf.

Rhizoctonia solani is a collective species comprising at least 13 anastomosis groups (AGs). As different AGs differ in their host range, epidemiology and fungicide sensitivity, knowledge about AG distribution is essential for disease prediction and management. AG-3 is the major cause of Rhizoctonia diseases of potato. Black scurf is almost exclusively associated with this AG (1). Other AGs, including AG-2-1, AG-4, AG-5 and AG-8 (1), have also been reported to be associated with potato diseases from different parts of the world.

In this study, we have collected 50 isolates of R. solani from potato in New Zealand, and report here the identification and characterisation of AG-2-1 isolates.

MATERIALS AND METHODS

Collection and isolation
Tubers with sclerotia were collected from potato growing regions in New Zealand. Sclerotia were excised from tubers and grown on 2% water agar containing streptomycin sulphate. Tips of hyphae resembling R. solani were placed on 2% PDA for further growth, identification and storage.

AG determination
The following techniques were used to confirm the AG of the isolates:

- qPCR: Total genomic DNA from isolates was extracted and used as a template for qPCR using AG specific primer pairs (South Australian Research and Development Institute; confidential agreement).
- DNA sequencing: PCR amplification and sequencing of the internal transcribed spacer region (ITS) were carried out with primer pair ITS4 and ITS5. Consensus sequences were analysed with BLAST. Phylogenies were constructed using PAUP.
- Anastomosis test: Isolates were each plated on PDA with available AG tester strains, as well as with each other, to determine anastomosis capability.

Determination of pathogenicity
Glasshouse and shade house experiments were carried out to investigate effects of isolates on potato plant (cv. Ilam Hardy) root growth, stolon infection and tuber yield, and on incidence and severity of R. solani sclerotium formation on tubers. Both experiments were laid out as Latinized resolved row/column designs. Data were analysed with mixed models (fitted with restricted maximum likelihood (3)) that included adjustments for any spatial effects patterns.

RESULTS

qPCR analysis and ITS DNA sequencing identified 45 isolates as AG-3, four as AG-2-1 and one as AG-4. Anastomosis tests showed the presence of significant clonal variation in AG-3 (dividing the isolates into 20 groups), whereas AG-2-1 isolates were all compatible.

In pathogenicity experiments, AG-2-1 isolates fell into two groups based on virulence. Group 1 gave high stolon disease scores (Fig. 1), caused severe tuber deformities, and produced sclerotia only on tuber initials but not on larger tubers. Group 2 isolates, including the AG-2-1 tester isolate, produced less disease. They did not damage stolons or cause tuber deformation, but formed moderate amounts of black scurf on large tubers.

Figure 1. Mean stolon disease scores from R. solani isolates. ▲ indicate AG-2-1 isolates of Group 1; ★ indicate AG-2-1 isolates of Group 2. ○ indicates the experimental negative control (no R. solani). Error bar shows 95% confidence limits for any mean. Dotted line shows the approximate point where scores become significantly greater (P=0.05) than the negative control.

DISCUSSION

AG-3 is the most predominant AG on potatoes in New Zealand followed by AG-2-1. Two distinct groups of AG-2-1 isolates were found in this study. The highly virulent Group 1 isolates gave severe infection and tuber malformation, whereas Group 2 isolates were largely avirulent. Woodhall et al. (2) found similar variation in pathogenicity amongst AG-2-1 isolates, and successfully correlated those groups with specific IGS1 sequence length. We are conducting IGS1 sequencing to examine whether a similar correlation exists in AG-2-1 populations from New Zealand.

ACKNOWLEDGEMENTS

This research was funded by Potatoes New Zealand and the NZ Foundation for Research, Science and Technology (Contract LINX0804).

REFERENCES
RESISTANT VARIETIES AS A MANAGEMENT TOOL FOR THE POTATO CYST NEMATODE (GLOBODERA ROSTOCHIENSIS) IN VICTORIA, AUSTRALIA.


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INTRODUCTION

Potato cyst nematode (PCN) is a serious quarantine pest of potatoes world-wide. Globodera rostochiensis, one of two species of Globodera, was first detected in the state of Victoria in 1991 (1). Its distribution is restricted to several properties east of Melbourne that are subject to quarantine control.

Although some farmers have grown potatoes on infested sites for nearly 20 years, options for managing the nematode, apart from regulation, have not been explored by government agencies or by industry. This paper reports on the results of a trial on a PCN infested site at Gembrook that compares the effects of susceptible and resistant varieties on yield and on PCN cyst numbers in soil. There is evidence of a greater abundance of PCN and reduced potato productivity in this area with the frequent cropping of susceptible varieties.

MATERIALS AND METHODS

Trial design was a randomised block with susceptible cvs. Sebago, Coliban (ware) and Trent (crisping) and resistant cvs. Nicola, Crop 13 (ware) and Atlantic (crisping) each planted in 10 m long-two row plots, replicated 6 times. Certified seed potatoes of each variety were planted in late November 2008. The crop was managed by the grower as per an adjacent potato crop. The middle seven meters of each plot was harvested in late June 2009 and tubers weighed into unmarketable (<120 g, > 450 g and damaged) and marketable (120-450 g) ware categories.

A 500 g soil sample was taken from each plot prior to planting and again after harvest (40 cores, 15 mm diameter by 100 mm deep/plot). PCN cysts were extracted from the soil samples using standard methods (2). Differences in cyst numbers between varieties at harvest were analysed by ANOVA with the number of cysts at planting as a covariate.

RESULTS AND DISCUSSION

An abundance of PCN cysts were evident on the roots of mature plants of the susceptible cvs. Trent, Sebago and Coliban, but were not apparent on the roots of the resistant cvs. Atlantic, Crop 13 and Nicola.

Plot cyst numbers ranged from 96 to 1107 cysts/500 g dried soil prior to planting (mean 544/500 g), and 114 to 1278 cysts/500 g at harvest (623/500 g). Contrary to published literature, there was no evidence of significant reductions in cyst numbers following one season of resistant cultivars. Cysts were more numerous (P<0.001) after Coliban and Sebago than after the three resistant varieties, whereas counts after Trent were intermediate between the resistant and the other two susceptible cultivars (Fig. 1).

Trent and Sebago produced less (P<0.001) marketable tubers than Atlantic, Crop 13, Nicola and Coliban (Fig. 2). The susceptible Coliban appeared better able to tolerate damage caused by nematode than Trent and Sebago.

This study indicates that resistant cultivars promise better yields on highly infested sites and have potential for managing cyst numbers on lightly infested sites, which are more typical of most PCN infestations in Victoria.

ACKNOWLEDGEMENTS

The Victorian Department of Primary Industries funded this work. Seed potatoes of cv. Crop 13 were used with permission of Plant and Food Research New Zealand. The help and generosity of the potato grower on whose farm the trial was conducted is gratefully acknowledged.

REFERENCES

INTRODUCTION
In Victoria, soilborne pathogens (including Sclerotinia, Pythium, Fusarium and Rhizoctonia spp.) cause significant crop losses in vegetable production. Crop rotation, biofumigation and improving soil health are IPM compatible strategies reported to reduce the impact of some soilborne diseases (1,2). This paper reports on preliminary results from laboratory and the first season’s field trials being conducted to investigate the potential of these strategies for managing soilborne diseases in vegetable production systems in Victoria, Australia.

MATERIALS AND METHODS

In-vitro screening
Root and shoot tissue from three Brassica cultivars (Table 1) grown in replicated field trials was freeze dried and added (0.25 or 0.5 g/plate) to one side of split Petri dishes. Mycelial growth of four soilborne pathogens (Table 1) was measured on the other half of the plate on PDA. The control potential of treatments was expressed as a percentage inhibition of growth or complete kill (biocidal effect) compared to untreated controls. Fumafert™, a Brassica seed meal, was used as a positive control. Another ten Brassica cultivar/species are currently being screened for their biofumigant potential in vitro and in pot trials.

Long-term field trials
Four trials were established on commercial farms in 2008/9. Data from one trial (Lindenow, Victoria) is reported here.

Break crop treatments (Table 2) were arranged in a randomised block design with six replicate plots (6 by 38 m) per treatment. Break crops, sown during March 2009, were pulsed and incorporated when Brassica plants were at 80% flowering in June (Mustclean™) and July 2009. Green beans were sown in September 2009. Disease and yield assessments were conducted at commercial harvest (Dec 2009) from a 1 m² area of each plot. Root rot severity was visually assessed using a 0-5 scale. The effect of treatments on a range of other parameters (e.g., soil chemical and physical properties) was measured, but is not reported here.

Glucosinolate (GSL) and isothiocyanate (ITC) analysis
Brassica shoots were collected from replicated field trials prior to crop incorporation. The tissue was stored frozen (-20°C) before being freeze dried and analysed for GSL levels by HPLC. Soil samples were collected four hours after Brassica treatments were incorporated and stored frozen (-20°C) until GLC analysis for ITC compounds.

RESULTS AND DISCUSSION
Caliente 199™ was the most effective biofumigant treatment in-vitro, significantly reducing growth of S. minor, P. dissotocum (complex), F. oxysporum and R. solani and killing mycelium of these pathogens at the highest rate 0.5 g/plate (Table 1). BQ Mulch™ also inhibited growth of all pathogens, but was not biocidal. Mustclean™ was inhibitory only to P. dissotocum (complex) at the highest rate used (Table 1). Caliente 199™ had the highest average concentration of shoot GSL (2-propenyl) across all field sites although the concentration recorded in tissue collected at Lindenow was much lower than the other sites (Table 2). This may be due to uneven crop growth across the plots due to soil fertility.

In the trial at Lindenow, all biofumigant and legume break crops significantly reduced (P<0.05) the severity of root rots in the subsequent green bean crop compared to fallow and cereal/grass crops. These preliminary results indicate potential disease control benefits for this cropping system/site and warrant further investigation. Sclerotinia levels were too low to allow treatment comparison.

Table 1. In-vitro effect of biofumigant treatments on mycelial growth of four soil-borne pathogens compared to untreated control.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>S.m</th>
<th>P.d</th>
<th>F.o</th>
<th>R.s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fumafert™</td>
<td>0.25</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Caliente 199™</td>
<td>0.25</td>
<td>I</td>
<td>I</td>
<td>B</td>
</tr>
<tr>
<td>Muscleteal™</td>
<td>0.25</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>BQ Mulch™</td>
<td>0.25</td>
<td>I</td>
<td>I</td>
<td>N</td>
</tr>
</tbody>
</table>

Table 2. Effect of crop rotation on GSL, ITCs, root rot severity and yield of green beans at Lindenow, Victoria.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Leaf GSLa</th>
<th>ITCs soilb</th>
<th>Root rot severity</th>
<th>Yield (kg/m2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caliente 199™</td>
<td>28.2 ± (53.2)</td>
<td>0.194 a</td>
<td>2.1 c</td>
<td>0.90</td>
</tr>
<tr>
<td>Mustclean™</td>
<td>30.6 ± (25.4)</td>
<td>0.713 b</td>
<td>1.9 c</td>
<td>0.72</td>
</tr>
<tr>
<td>BQ Mulch™</td>
<td>25.8 ± (29.9)</td>
<td>0.556 b</td>
<td>2.3 bc</td>
<td>0.80</td>
</tr>
<tr>
<td>Faba bean</td>
<td>-</td>
<td>-</td>
<td>1.8 c</td>
<td>0.84</td>
</tr>
<tr>
<td>Vetch</td>
<td>-</td>
<td>-</td>
<td>2.2 bc</td>
<td>0.81</td>
</tr>
<tr>
<td>Ryegrass</td>
<td>-</td>
<td>-</td>
<td>2.9 a</td>
<td>0.78</td>
</tr>
<tr>
<td>Trictele</td>
<td>-</td>
<td>-</td>
<td>2.8 ab</td>
<td>0.80</td>
</tr>
<tr>
<td>Fallow</td>
<td>-</td>
<td>0.000</td>
<td>2.9 a</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Means in a column with different letters are significantly different (P<0.05).

REFERENCES
INTRODUCTION

*Fusarium culmorum* is the dominant causal species of crown rot (CR) in Turkey and is reported to causes losses up to 43% on winter wheats. In rainfed wheat production systems where cereal monoculture is practiced extensively such as in Turkey, rotation offers limited opportunity to control Crown Rot. As a result, efforts have been made to identify resistant wheat cultivars for Turkey and the West Asia, North Africa region.

MATERIALS AND METHODS

121 spring and winter wheat cultivars were screened for their resistance to CR under inoculated field (F) and greenhouse (G) conditions for two consecutive years. Of these, 28 putatively resistant cultivars were further evaluated in both F and G against moderately resistance (MR) and susceptible (S) check cultivars.

Greenhouse screening technique

Surface sterilized pre-germinated seeds (3-4 days old) were soaked (1) for 1 minute in a 1x10^3/ml spore suspension of *F. culmorum* isolate F2. Seven replicates of each cultivar were grown in separate tubes (12 x 2.5 cm) filled with an autoclaved standard potting mix (70 sand:29 field soil:1 organic matter) arranged as Randomised Complete Block Design. Plants were grown in a greenhouse with 12 h light, at 24±3°C and harvested 8 weeks post inoculation. Crowns were given scores for disease browning using a slightly modified rating method (2), from the base to 10 cm of each tiller based on browning (0: 0%, 1: 1-10%, 2: 10-25%, 3: 25-50%, 4: 50-75%, 5: >75%).

Field Screening technique

Three 20g seed packets of each of the 28 wheat cultivars were soaked for approximately 60 seconds in an 3.2x10^3 spore/ml solution of the monosporic *F. culmorum* isolate F2, and left to air dry. Three non inoculated 20g seed packets of each cultivar were also prepared. Using a lattice design, three replicates of each cultivar were planted side by side (non-inoc vs inoc) in 1.5 m rows under natural rainfed field conditions in Konya, Turkey. Seed were planted in October and plants harvested in July. Depending on the symptoms, the key characteristic symptom of CR, the formation of White Heads (WH) were scored at the ripening stage (Zadock growth scale 91-94) on a 0-5 scale (0: No WH, 1: 5-10%, 2: 10-29%, 3: 30-69% 4: 70-89% 5: >90-99%) comparing with and without inoculated plots. Additionally around similar time 30 tillers from inoculated WH and CS were sampled and Crown Score (CS) was given for each tiller based on browning using same scale as Greenhouse, however scored from base to 15 cm.

RESULTS and DISCUSSION

There was a significant cultivar effect for all data sets (P<0.05). Significant correlations (P=0.05) were found between F years of screening for both WH and CS combinations, and also with F and G data. However these correlations were not considered to be very strong with correlation coefficients (r) between 0.39-0.50.

This lack of strong correlation has also been reported by others, and is clearly known that environmental factors (particularly water stress post anthesis) play an important role in the expression of key crown symptoms such as WHs. One of the challenges to this work is the inherent field variation between replicates.

Four of the 28 putatively resistant cultivars consistently performed similarly to the MR check cultivars (2-49, Sunco) and were classified MR. Two are high yielding winter wheat breeding cultivars originating from the joint Turkey-CIMMYT-ICARDA International Winter Wheat Improvement Program and two are Australian spring wheat breeding cultivars previously found to be MR under field conditions (Lu, pers.com). This work clearly illustrates the difficulty in identifying valuable sources of resistance MR to CR, and the fact that multiple years of data are required to be confident in the results. These 4 identified lines are considered to be very valuable for bread wheat improvement globally for CR. CIMMYT in collaboration with Turkey is happy to collaborate with other colleagues on CR and also share promising germplasm.

ACKNOWLEDGEMENTS

Anatolian Agriculture Research Institute of Eskisehir, and Bahri Dagdas Institute Konya Turkey are thanked for providing technical and statistical support (Mr E Savaslı, Dr N Botal, Ms A Yorgancilar, Mr A T Kilinc and Mr F Ozdemir). Dr Meqin (Australian Grain Technologies Australia) are thanked for supply of SW lines. Dr Dababat is thanked for review of manuscript and GRDC for financial support.

REFERENCES


WHEAT GENETIC RESISTANCE TO DRYLAND CROWN ROT (*FUSARIUM CULMORUM*) FROM INVITRO SEEDLING AND ADULT PLANT SCREENING

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Figure 1. Confirmed MR Crown Rot wheat lines from multiple years of field (F) and one year greenhouse (G) screening. Bars represent SED between data sets, Y axis refers to disease assessment of CR (found in materials and methods).
INTRODUCTION
Quantification of disease severity is essential for understanding plant-pathogen interactions. Disease severity is generally measured using visual assessment of symptoms, as these techniques are relatively quick and inexpensive. For each application the approach used varies, the final choice depending on the disease being studied and the desired outcomes of the research program.

Stem browning and, less commonly, whitehead formation are used to screen cereals for resistance to crown rot (caused by Fusarium spp.) under controlled conditions. The expression of these visual symptoms is greatly affected by seasonal conditions (1) which raises questions about their validity for use in resistance screening under field conditions.

This study explores the value of visual assessment of disease incidence, stem browning and whiteheads for screening durum and bread wheats for resistance to F. pseudograminearum under field conditions.

MATERIALS AND METHODS
A subset of information from 6 site-year combinations (referred to here as sites) used in screening for crown rot resistance was assembled in a data base covering 13 durum and bread wheat entries. Field experiments were undertaken in South Australia in the Murray Mallee (Cambrai: 2006 - naturalised inoculum), Mid North (Hart: 2006, 2007, 2008 - inoculated seed) and Lower North (Mallala: 2007, 2008 - inoculated seed).

The plants along one side of a 0.5 m ruler were taken from 4 points in each plot at mid to late grain fill. Whiteheads and total heads were counted to determine whitehead %. Main stems were stripped of leaf sheaths and scored on a visual scale where 0=0%; 1=1-10%; 2=10-25%; 3=25-50%; 4=50-75% and 5=>75% of browning from the sub-crown to the first node. Disease incidence = (number of main stems with browning/total number of main stems)*100.

Data were interrogated using the ANOVA and Summary Statistics (correlation) options in GenStat.

RESULTS AND DISCUSSION
All disease assessment variables detected significant differences among entries (Table 1). For disease incidence and stem browning this is consistent with the suggestion that the main expression of resistance to crown rot is in the rate of development of discoloured tissue (2). As expected, all variables detected significant differences among sites (Table 1).

Stem browning discriminated somewhat better among entries (had a higher v.r.) than did the other variables (Table 1). This implies that browning score is likely to be the more powerful tool for resistance screening. However, there was a small but significant interaction between site and entry for this variable (Table 1), which may help explain why selecting resistant material using this variable has proven to be difficult.

Disease incidence did not discriminate better (or worse) among sites than stem browning and there was also a weak site by entry interaction for this variable (Table 1). Neither of these findings is consistent with reports in the literature that crown rot incidence is greatly affected by soil moisture but not by host cultivar (1). This may be due to the use of visual symptoms in this study rather than plating plant tissues on to agar to assess disease incidence.

Table 1. Ability of stem browning, visual disease incidence and whitehead % to discriminate among entries and sites (v.r. = variance ratio)

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>v.r.</th>
<th>F prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem browning – entry</td>
<td>12</td>
<td>26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Disease incidence – entry</td>
<td>12</td>
<td>16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Whitehead % - entry</td>
<td>12</td>
<td>18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stem browning – site</td>
<td>5</td>
<td>105</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Disease incidence – site</td>
<td>5</td>
<td>101</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Whitehead % - site</td>
<td>5</td>
<td>58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stem browning – site:entry</td>
<td>60</td>
<td>1.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Disease incidence - site:entry</td>
<td>60</td>
<td>1.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Whitehead % - site:entry</td>
<td>60</td>
<td>3.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Whitehead % discriminated well among entries but less well among sites and was more sensitive to site by entry interaction than the other variables (Table 1). The larger site by entry interaction for whiteheads may at least partly explain why whitehead % was less able to discriminate between sites than the other variables.

Simple correlation coefficients (c.c.) show that whiteheads and stem browning (c.c. = 0.74) and whiteheads and disease incidence (c.c. = 0.58) have a good relationship but are not measuring quite the same thing. That the c.c’s are not closer to 1.00 is likely to be at least partly due to genotype by environment interactions as well as, perhaps, being influenced by tolerance.

None of the visual symptoms assessed here would provide a consistent ranking of cereals in different experiments. Large numbers of field experiments or earlier sampling times may resolve this issue as may alternative methods of resistance assessment (e.g. fungal DNA concentrations in plant tissue). However, it may need to be accepted that field screening will encompass resistance, tolerance and agronomic adaptation to environment.

ACKNOWLEDGEMENTS
The authors thank Chris Dyson for statistical and Greg Naglis, Mark Butt and Jim Lewis for technical assistance. Financial support was provided by SARDI and GRDC (DAS00032; DAS00073).

REFERENCES
ELEVATED ZINC AND MANGANESE LEVELS GIVE MODERATE REDUCTIONS IN SPONGOSPORA SUBTERRANEA INFECTION OF POTATO ROOTS

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INTRODUCTION

Spongospora subterranea f. sp. subterranea causes powdery scab of potato tubers (Solanum tuberosum). This disease is important where crops are grown under intensive management (especially high fertility and irrigation). Powdery scab lesions on tubers cause severe quality reductions of seed, fresh market and processing potatoes (1). The pathogen also causes galls on roots and stolons, and root infection reduces plant productivity by disrupting water and nutrient uptake (1). Manipulation of soil nutrients could be part of integrated powdery scab management (2), and the pesticide mancozeb (which contains zinc (Zn) and manganese (Mn)) can reduce powdery scab in field-grown potatoes (see 2).

An experiment was carried out to measure effects of different amounts of Zn or Mn on infection of potato roots by S. subterranea. Tested amounts of the elements were below, similar to and above levels commonly found in field soils. The study aimed to indicate potential for Zn or Mn soil amendments in powdery scab control.

RESULTS AND DISCUSSION

Table 1 presents means of plant and root parameters and numbers of galls by 40-50% compared with the lowest rates of elements tested.

Table 1: Mean plant parameters and numbers of S. subterranea root galls (/g root dry weight) for potato plants grown in sand infected with standard nutrient solution (Control), or solution modified with different amounts of Zn or Mn.

<table>
<thead>
<tr>
<th>Amount (µg/ml)</th>
<th>Shoot dry weight (g)</th>
<th>Root dry weight (g)</th>
<th>Number of galls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.66</td>
<td>0.69</td>
<td>96</td>
</tr>
<tr>
<td>Zn 0.01</td>
<td>1.68</td>
<td>0.82</td>
<td>78</td>
</tr>
<tr>
<td>0.03</td>
<td>1.51</td>
<td>0.82</td>
<td>78</td>
</tr>
<tr>
<td>0.25</td>
<td>1.72</td>
<td>0.85</td>
<td>76</td>
</tr>
<tr>
<td>0.50</td>
<td>1.48</td>
<td>0.85</td>
<td>62</td>
</tr>
<tr>
<td>1.0</td>
<td>1.75</td>
<td>0.86</td>
<td>49</td>
</tr>
<tr>
<td>2.0</td>
<td>1.68</td>
<td>0.82</td>
<td>64</td>
</tr>
<tr>
<td>Mn 0.05</td>
<td>1.61</td>
<td>0.75</td>
<td>92</td>
</tr>
<tr>
<td>Control</td>
<td>1.68</td>
<td>0.82</td>
<td>78</td>
</tr>
<tr>
<td>0.25</td>
<td>1.65</td>
<td>0.83</td>
<td>73</td>
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<tr>
<td>1.0</td>
<td>1.82</td>
<td>1.05</td>
<td>53</td>
</tr>
<tr>
<td>2.0</td>
<td>1.78</td>
<td>1.03</td>
<td>54</td>
</tr>
<tr>
<td>5.0</td>
<td>1.79</td>
<td>0.95</td>
<td>54</td>
</tr>
<tr>
<td>10.0</td>
<td>1.60</td>
<td>0.78</td>
<td>70</td>
</tr>
</tbody>
</table>

LSD (F = 0.05) 0.27 d.f. 83 84 84

These results demonstrate that elevated levels of Zn or Mn have moderate effects on S. subterranea infection of potato roots. Furthermore, root galling was severe even at high rates of both elements. These results suggest that Zn or Mn soil amendments may not reduce powdery scab to acceptable levels in potatoes grown in fields infested with S. subterranea.

ACKNOWLEDGEMENTS

The NZ Foundation for Research Science and Technology and HAL (through the Australian Processing Potato Research Programme) funded this research.

REFERENCES


ARE ORGANIC FARMING SOILS MORE DISEASE SUPPRESSIVE?

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INTRODUCTION

Due to lack of diversity, commercial banana plantations are susceptible to pests and diseases. Organic agriculture is a way of farming that aims to develop holistic management in an ecological way. Organic agriculture tries to promote disease suppression through healthy soils by increasing biological activity and diversity. This is achieved through the application of organic fertilizers and increasing organic inputs to stimulate soil microbial biomass and activity (1).

Fusarium wilt of bananas caused by Fusarium oxysporum f. sp. cubense (Foc), also known as Panama disease, has been a devastating disease throughout the world. So far, no fungicides or cultural measures have been found that sufficiently control Foc.

The aim of this research was to assess whether organic farming systems and soils are more resilient than inorganic farming systems to soilborne diseases, in particular Fusarium wilt.

MATERIALS AND METHODS

Survey A survey was conducted to compare five organic and five conventional banana plantations in paired sites. Soil samples were collected and analysed for chemical, physical and biological soil health indicators (2). Disease development of F. oxysporum f. sp. lycopersici in tomatoes and Foc in bananas was studied in pot trials as a possible test for identifying Fusarium suppressive soils. Disease progress was recorded once a week. The area under the disease progress curve (AUC) for each plant was calculated. The soil indicators and AUC values were analysed by one-way-ANOVA and significant means were tested with Fisher’s protected LSD test.

Field experiment A field experiment was established on a Ducasse plantation infested with Foc Race 1. The experiment had five treatments: 1, a combination of two Effective Microbes based inoculants (EM) on fresh compost; 2, aged compost; 3, Natural Silica (ground diatomaceous earth); 4, a combination of all treatments; and 5, untreated control. The EM inoculum was reapplied fortnightly. The experiment was managed organically according to BFA standards. Disease progress was recorded every two weeks and growth was measured once a month. Soil samples were taken after two months and at the end of the experiment at four months post application. Soil samples were analysed on pH, EC, labile Carbon, FDA, β-glucosidase, nematode community structure. The same statistical analyses were used as described previously.

RESULTS AND DISCUSSION

Survey Soils from organic farms had consistently higher scores on biological soil health indicators than conventional farms (Table 1). In initial trials with tomatoes no disease was observed, possibly due to low pathogenicity of the Fusarium strain. Repeated tomato bioassays were complicated by additional pathogens present in the soil, such as Pythium spp., Rhizoctonia, and Ralstonia. General plant health in tomatoes was positively correlated to nematode diversity and magnesium and negatively correlated to potassium.

Field experiment At 2 months post application, treatment 4 scored consistently and statistically higher on key soil health indicators; pH, FDA, β-glucosidase, nematode diversity, and proportions of fungal feeding nematodes and lower on plant-parasitic nematodes than untreated control. Treatment 4 also had a significantly lower AUC based on wilting symptoms than untreated control (Figure 1). Plant death was correlated to FDA, nematode diversity, percentage of fungal feeding nematodes and bacterial : fungal feeding nematode ratio.

This work demonstrates that organic farming systems increase overall biological activity and diversity and reduce the amount of plant parasitic nematodes in banana soils. Further work should be undertaken to determine whether this is simply due to organic fertilizers or due to more complex organic farm management. The field trial has shown that organic amendments can reduce Foc incidence in a short time. Nematode diversity seems to be an important indicator for plant health in both the survey and the field trial.

ACKNOWLEDGEMENTS

We want to thank Peter and Vivien Grant for providing the field site, Sharon Hamil for tissue culture plants and Ken Bellamy for providing the EM products. This project was funded by ACIAR and DEEDI.

REFERENCES


Table 1. Values of key soil health indicators in organic and conventional banana soils.

<table>
<thead>
<tr>
<th>System</th>
<th>Labile C</th>
<th>FDA</th>
<th>β-glucosidase</th>
<th>Plant parasitic nematodes %</th>
<th>Non feeding nematodes %</th>
<th>Nematode diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic</td>
<td>0.622</td>
<td>1.306</td>
<td>548</td>
<td>31</td>
<td>14</td>
<td>2.05</td>
</tr>
<tr>
<td>Conventional</td>
<td>0.374</td>
<td>0.847</td>
<td>345</td>
<td>73</td>
<td>3</td>
<td>1.47</td>
</tr>
<tr>
<td>LSD</td>
<td>0.03</td>
<td>0.006</td>
<td>0.043</td>
<td>0.02</td>
<td>0.05</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Figure 1. Disease progress curve of field banana plants. Disease marker displayed is average disease value per treatment. ○ is aged compost, △ is silicon, ● is control, ◊ is EM and X is the combination treatment.
INTRODUCTION

Spongospora subterranea f. sp. subterranea (Sss) is a soilborne biotrophic protozoan pathogen, which causes powdery scab of potato (Solanum tuberosum). This is an economically important disease, causing severe losses in all types of potato production, and in most potato growing regions. Sss is capable of long term survival (many years) by producing aggregations of resting spores in sporosori. There are no completely effective control methods for the disease. Current information on powdery scab and Sss has recently been reviewed (1).

Plant pathogens are capable of constant adaptation to changes in agricultural ecosystems (2). Locally occurring virulent pathogen mutants can infect plants in monocultures, increasing in frequency through selection and rapidly spreading over large areas and continents. Effective control strategies must therefore target pathogen populations rather than individuals.

Potato cultivars resistant to powdery scab are likely to provide sustainable control of the disease. For most pathogens, breeders screen new host lines against a range of pathogen strains representing known genetic diversity to select for durable resistance. However, there is minimal information about the genetic variation of Sss or the role of sexual recombination in its life cycle.

The present research aims to determine the amount of genetic variation among and within populations of Sss. Specific microsatellite markers were obtained and applied to seven field populations of the pathogen, to examine evolutionary history and potential to evolve.

METHODS

Sample collection. Seven populations of Sss were examined. Sporosorus material was from tuber lesions from Australia (20 samples), New Zealand (40 samples), Norway (25 samples) and Switzerland (two populations, 22 and 25 samples), and root galls from Colombia (two populations, 8 and 5 samples). One sample of Spongospora subterranea f. sp. nasturtii (Ssn: crook root of water cress) was also analysed.

DNA extractions from each dry sample (up to 200 mg) were performed using the CTAB protocol (3). Microsatellite library. Four different specific microsatellite loci were isolated from Sss DNA (4). Primers for the different loci were designed using primer3® version 0.4.0 (5).

Fragment analysis. The microsatellite loci were amplified by PCR using fluorescent labelled primers. Labelled PCR products were processed with a 3730xl DNA Analyzer (Applied Biosystems). The sequencer files were analysed with the GeneMapper® 4.0 Software to size and genotype the alleles.

RESULTS AND DISCUSSION

No variation was detected between and within populations, neither from different countries nor between continents. The only exceptions were the samples from Colombia that showed microsatellite polymorphism. The number and position of detected alleles was the same in all samples from Colombia, indicating that there is no variation within and between these populations. The same was observed for the other populations, although the number and position of detected alleles varied compared to the Colombian samples (Table 1).

Table 1. Microsatellite locus name, core sequence and expected amplicon size, allele size range and number of alleles from Colombian populations (C), the five other populations (O), and total (T).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Core sequence</th>
<th>Size (bp)*</th>
<th>Allele size range†</th>
<th>No. of alleles C</th>
<th>O</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Msat6</td>
<td>GAC/CAC</td>
<td>210</td>
<td>195-239</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Msat45</td>
<td>TCA</td>
<td>232</td>
<td>194-270</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Msat103</td>
<td>CT</td>
<td>199</td>
<td>194-200</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Msat104</td>
<td>GA</td>
<td>265</td>
<td>195-269</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

*Size of amplicon based on actual sequence data
†Allele sizes based on GeneMapper® output, internal size standard: LIZ 500 (Applied Biosystems).

Additionally, the Ssn sample showed the same pattern as most of the Sss populations and was also different from the Colombian samples. The fragment analysis results indicate that there may be less variation among Sss populations than expected. The results also raise the questions of whether Sss is really a distinct forma specialis, or if similarity was due to contamination. Hypotheses explaining the contrasting results for Colombia could be geneflow between distinct populations or the presence of another species or forma specialis. Additional microsatellite loci and more populations, especially from South America, will need to be examined to distinguish between these and other hypotheses. Further fragment analyses are currently being performed.

ACKNOWLEDGEMENTS

This project is funded by Horticulture Australia Ltd, The United Kingdom Potato Council, The New Zealand Foundation for Research Science and Technology, Horticulture New Zealand and the ETH Zürich.

REFERENCES

SUPPRESSION OF DAMPING-OFF OF RADISH CAUSED BY RHIZOCTONIA SOLANI AG2.1 WITH SOIL CARBON AMENDMENTS

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INTRODUCTION
Rhizoctonia solani (Rs) is a necrotrophic soil-borne pathogen with a high competitive saprophytic ability. The fungus is one of the most important causal agents of damping-off of Brassicaceae crops in Australia. The use and decomposition of carbon amendments (CA) in soil plays an important role in plant-pathogen interactions, pathogen survival and soil suppressiveness (1). Organic matter may increase populations of saprophytic microorganisms in soil, which can act as antagonists to plant pathogens (2). Incorporation of various CA into Rs-infested soil has been shown to suppress disease in several studies (1, 2). The use of CA in the vegetable industry is considered an important component of soil health management, and may have implications for carbon sequestration into soils under future carbon pollution reduction schemes.

The aim of this study was to evaluate and screen established (compost and humate) and emerging (biochar and lignite) CA for suppression of damping-off of radish caused by Rs (AG 2.1).

MATERIALS AND METHODS
A fine sandy clay loam soil (pH 6.4) was collected from a commercial vegetable farm in Deavon Meadows, Victoria. Four agar plates (WA) containing Rs AG2.1 (1 wk-old) were blended with 250 ml of sterile distilled water, and used to inoculate 8 kg of soil. Following inoculation, soil was amended with biochar, lignite, compost and humate at a rate of 650 mg of C / g dry soil (equivalent to 100 t/ha of biochar). Treatments were balanced for the volume of the amendment added to soil (using washed river sand), and for nitrogen content. Controls consisted of unamended soil, and soil fumigated with Dazomet (10 mg/g of soil).

After a resting period of 2 weeks, soils were potted (16.5 cm diameter), sown with 10 seeds of radish (Raphanus sativus, cv. French Breakfast), and placed in a glasshouse. Three weeks after sowing, disease incidence, soil pH, and microbial activity were determined using fluorescein diacetate hyrolysis (FDA). DNA was extracted from soil using an UltraClean Soil DNA Isolation Kit, and the concentration of Rs (AG 2.1) was determined using standard quantitative detection method techniques. Seedlings were then removed, soils re-sown with a second rotation of radish, and measurements repeated after 3 weeks. There were a total of three rotations in the trial.

The trial was conducted as randomised complete block design with six blocks. Data was analysed using ANOVA in GenStat (v. 11).

RESULTS
Adding CA to soils infested with Rs gave intermediate levels (2 - 28% lower disease incidence compared with unamended soils) of suppression of damping-off in radish (Fig. 1). The fumigant Dazomet significantly reduced disease incidence by c. 30% across all rotations. By comparison, disease incidence in soils treated with CA was not significantly different from those in fumigated soils. However, only humate significantly reduced disease incidence, compared with unamended soils by the final rotation.

Concentrations of Rs DNA tended to increase in soils treated with CA, compared with unamended soils. By the third rotation, there was between 378 and 680 pg Rs DNA/g soil in all the CA soils, which was significantly higher than that in the unamended control (123 pg Rs DNA/g soil). Similarly, soil pH (8.59 in humate-treated soils) and FDA (5.59 and 8.55 μg fl./g soil in compost and lignite-treated soils, respectively) tended to increase in CA-treated soils compared with unamended soils (FDA = 2.80 μg fl./g soil). There was no correlation between pH or FDA with disease incidence.

Figure 1. Effect soil carbon amendments on radish damping-off. Bars with same letters are not significantly (p>0.05) different according to Student-Newman-Keuls multiple range test.

DISCUSSION
The use of CA has the capacity to improve soil health. The addition of CA neutralised soil pH, and increased soil biological activity and carbon content. Whilst CA increased the biomass of the saprophytic pathogen Rs in soil, it also gave moderate suppression of damping-off of radish. This opposing effect may be due to an increase in specific groups of soil microflora by CA, which interfere with pathogenesis through antagonism (3), or stimulation of disease resistance in the host.

The balance between increased biomass of saprophytic pathogens and improved disease suppression may also be affected by the form of CA added to soil. Rs produces enzymes that allow it to utilise cellulose-rich substrates (2). Therefore, it was expected that Rs biomass increased in compost-amended soils compared to soils treated with inert carbon sources (e.g. biochar). Even the source of biochar may affect the outcome of pathogenesis. In the current trial, biochar from green waste caused moderate disease suppression, but biochar from rice hulls used in a previous trial increased the incidence of damping-off from 40 to 80%.

CA represents an important tool for improving soil health and carbon sequestration into soils. The challenge for pathology is to better understand the mechanisms that moderate the balance between pathogen build up and soil suppressiveness for more reliable disease management.

REFERENCES
RESPONSE OF SOIL MICROFLORAL COMMUNITIES TO STUBBLE ADDITION DIFFERS BETWEEN DISEASE SUPPRESSIVE AND NON-SUPPRESSIVE SOILS

INTRODUCTION
Biological suppression of soilborne diseases reported in southern Australian agricultural fields is a function of the population, activity and composition of the microbiota (microflora and microfauna) community (1). It is an inherent property of all biologically active soils but the level of suppression ability varies with edaphic and environmental variables. Management practices which supply higher biologically-available carbon inputs over long periods (greater than 5-7 years) can result in changes in the composition and activity of the soil microbial community and consequently support increased suppression (2). The majority of research on suppressive microbial communities in Australian soils has been done using just 'best-bets' by targeting individuals or groups of organisms mostly with culture based methods using selective media. However, efforts to reproduce disease control reliably through the introduction of individual bacterial and fungal inoculants in field environments have limited success. Our aim was to characterize the genetic diversity of bacteria and fungi and their response to carbon addition in soils with varying potential for biological disease suppression against Rhizoctonia bare patch in cereals.

MATERIALS AND METHODS
Surface (0-10 cm) soils were collected from fields at Avon, Waikerie and Streaky Bay in South Australia under multiple cereal crops and reduced till systems. Unamended control and soils amended with (i) carbon (C) (wheat stubble 0.5%w/w or 1% sucrose C) and (ii) Rhizoctonia solani AG8, were incubated at 15 oC for two weeks prior to microbial analyses and bioassay using wheat seedlings (3). Genetic diversity of bacteria and fungi was determined using 16S rDNA and ITS-PCR DGGE methods, respectively. Catabolic diversity of microbial communities was measured using carbon substrate utilization profiling. Root disease scoring was done on 4 week old seedlings.

RESULTS
The bioassay indicated highest disease suppressive potential in the Avon soil followed by Waikerie and the StreakyBay soil. Multivariate analysis of genetic diversity of bacteria, pseudomonads and soil fungi, and of C-substrate utilization profiles showed significant differences between the three soils, especially in terms of added C (e.g. stubble). Catabolic diversity and potential were generally lower in the Streaky Bay and Waikerie soils compared with the Avon soil.

DISCUSSION
Interactions between phytopathogenic fungi and soil bacterial and fungal communities influence the level of soilborne disease incidence. Our results suggest that in stubble treated soils (i) a diverse soil fungal community can help reduce pathogen effects and (ii) Pseudomonas species are one of the major drivers in biological control. Further in order to distinguish suppressive communities, these results demonstrate the importance of prior exposure of soils to factors, such as stubble retention, that contribute to the development of disease suppression.

ACKNOWLEDGEMENTS
Technical support was provided by M. Hicks and S. Kroker. CSIRO Entomology, Grains RDC and The Crawford fund, Australia provided financial support.

REFERENCES
TEMPORAL DYNAMICS OF RHIZOCTONIA SOLANI AG8 INOCULUM IN AUSTRALIAN SOILS

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INTRODUCTION
Rhizoctonia bare patch is a disease of seedlings caused by Rhizoctonia solani Kühn AG-8. Recent surveys indicate that it causes significant losses in cereals, $59 million pa, mainly in low to medium rainfall regions across southern Australia (1). While previous research has found the risk of yield loss can be reduced by management practices that increase seedling vigour, it remains a difficult disease to predict and control.

The incidence of Rhizoctonia bare patch has increased in recent years due to a significant increase in intensive cereals, reduced tillage and the higher frequency of drought years, particularly below average spring and summer rainfall. This has resulted in higher inoculum levels recorded in Predicta® tests prior to sowing during 2009.

R. solani fungus grows on soil organic matter and produces a hyphal network in the surface soil (2). Disease severity depends on the amount of Rhizoctonia inoculum, composition and activity of the soil biology community, available soil N levels over summer and at seeding and constraints to root growth (3). These complex relationships make it difficult to predict and manage this disease.

As part of a GRDC funded project we investigated the changes in inoculum, especially over summer, as influenced by environmental factors and soil biological activity under different rotation and tillage systems.

MATERIALS AND METHODS
During the 2008 off-season, surface soil (0-10cm) samples were collected from selected crop rotation and tillage treatments in field experiments at Waikerie (Alfisol) and Streaky Bay (Calcarosol) in SA and Galong (Red Brown soils) in NSW. Samples were collected at monthly intervals after crop harvest in 2008 until sowing in 2009.

Soils were analysed for R. solani AG8 DNA concentration (SARDI, RDTS), microbial activity, dissolved organic C and mineral N levels.

RESULTS and DISCUSSION
R. solani DNA levels at the start of 2008 crop season were 300, 160 and 100 pg / g soil at Waikerie, Streaky Bay and Galong sites, respectively. Changes in the concentration of R. solani DNA (pg / g soil) in soils following rotational crops.

Changes in AG8 DNA concentrations during summer 2009 at Waikerie (G) and Streaky Bay (S) show greater decline in Rhizoctonia DNA over summer compared with the other sites. Notice that at Waikerie the average decline was lowest at Waikerie (45%) compared with 70% reduction at Streaky Bay and Galong.


ACKNOWLEDGEMENTS
Financial support was provided by Grains RDC and host institutions of researchers.

REFERENCES

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BACTERIAL INOCULATION OF BANANA IMPROVES PLANT GROWTH UNDER REDUCED FERTILISER TREATMENT

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INTRODUCTION
High priorities for the Australian Banana industry are to manage diseases and improve soil health, which includes reducing fertiliser inputs and nutrient impacts on environmentally sensitive areas such as the Great Barrier Reef. Tissue cultured banana plants are used to prevent movement of disease and to improve farm efficiencies (1). However these plants show increased susceptibility to soil borne diseases such as Fusarium wilt when planted into infected soil. Controlled inoculation of tissue cultured plants with beneficial bacteria has potential to improve plant disease tolerance and reduce fertiliser requirement. To explore this concept, endophytic bacteria were isolated from within thousands of banana suckers. More than half (62%) of the bacteria belonged to genera reported to be beneficial to plants (2). In this study a small selection of isolates from nitrogen-fixing genera were evaluated for potential to improve growth of tissue cultured banana provided with low levels of fertiliser.

MATERIALS AND METHODS
Several isolates from four nitrogen-fixing genera, Herbaspirillum, Azospirillum, Azoarcus and Rhizobium, were selected from a bacterial population isolated from thousands of banana suckers. For further testing three control treatments (50%, 75% and 100% fertiliser) and sixteen bacterial treatments receiving only 50% fertiliser were applied to cv. ‘Williams’ tissue culture banana suckers. More than half (62%) of the bacteria belonged to genera reported to be beneficial to plants (2). In this study a small selection of isolates from nitrogen-fixing genera were evaluated for potential to improve growth of tissue cultured banana provided with low levels of fertiliser.

RESULTS AND DISCUSSION
Six of the sixteen bacterial isolates significantly improved growth of banana compared to the matched control provided with only half nutrient requirements (Table 1). Isolates 18 and 14 (Azospirillum sp.) and 10 (Herbaspirillum sp.) significantly increased shoot weight by 10 to 14% compared to the 50% control but did not match the weight of those plants given 75% or 100% fertiliser. Plants treated with isolates 6 (Herbaspirillum sp.) and 3 (Azoarcus sp.) were significantly taller than 50% control and the same height as plants given 75% fertiliser. Isolate 8 (Herbaspirillum sp.) significantly increased both height and fresh weight of treated plants. Root weight was not increased by any bacteria.

In hindsight it was overly ambitious to expect bacteria to overcome a 50% fertiliser deficit such that additional plant stress would have occurred perhaps confounding results. Future work will aim for optimal growth at a more realistic 30% fertiliser reduction target so that the plants can continue to grow but without added stress caused by nutrient deficiency.

<table>
<thead>
<tr>
<th>Fertiliser</th>
<th>Isolate</th>
<th>Shoot wt (g)</th>
<th>Stem Height (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>none</td>
<td>204.2 a</td>
<td>208.6 b</td>
</tr>
<tr>
<td>75%</td>
<td>none</td>
<td>156.0 b</td>
<td>185.7  b</td>
</tr>
<tr>
<td>50%</td>
<td>Isolate 18</td>
<td>116.0 c</td>
<td>168.7 bdef</td>
</tr>
<tr>
<td>50%</td>
<td>Isolate 14</td>
<td>116.2 c</td>
<td>175.3 bdef</td>
</tr>
<tr>
<td>50%</td>
<td>Isolate 10</td>
<td>117.7 c</td>
<td>173.6 bdef</td>
</tr>
<tr>
<td>50%</td>
<td>Isolate 8</td>
<td>113.8 cdef</td>
<td>180.1 b</td>
</tr>
<tr>
<td>50%</td>
<td>Isolate 6</td>
<td>109.09 cdef</td>
<td>183.05 bc</td>
</tr>
<tr>
<td>50%</td>
<td>Isolate 3</td>
<td>108.09 cdef</td>
<td>178.5  bde</td>
</tr>
<tr>
<td>50%</td>
<td>none</td>
<td>103.1  cdef</td>
<td>159.4  4e</td>
</tr>
</tbody>
</table>

Values followed by different letters indicate significant differences at p<0.05 according to LSD.

Tissue culture offers a controlled way to safely introduce bacteria providing the sterile plants with a resident endophytic population to improve plant performance. Some isolates could induce systemic resistance in plants improving their tolerance to disease while other isolates may improve the plant’s access to nutrients to reduce fertiliser requirements. In this preliminary work 40% of randomly selected bacterial isolates significantly improved growth under nutrient stress. In fact, all except one isolate delivered a higher mean shoot weight than the 50% control. These results justify further investigation of isolates and inoculation methods. Our large population of bacteria has now been characterised and isolates demonstrating attributes such as nitrogen fixation, phosphate solubilisation and pathogen inhibition will be targeted in future plant growth and pathogen challenge trials. Use of bacteria with demonstrated benefits to plant growth and defence should be considered as a strategy contributing to improved soil health.

REFERENCES
SPATIAL DISTRIBUTION OF THE SOIL BORNE PATHOGEN
COLLETOTRICHUM COCCODES AND SUBSEQUENT DISEASE EXPRESSION ON
POTATOES AT HARVEST

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INTRODUCTION
Black dot caused by the fungus *Colletotrichum coccodes*, is a major potato disease that causes significant economic losses due to downgrading of blemished tubers and reduced yields (1). Whilst the inoculum source for infection may be soil and/or seed, soil-borne inoculum is regarded as the more significant (2). Field experiments were conducted to evaluate a recently developed DNA based test to quantify the amount of *C. coccodes* in the soil and determine the risk of the disease developing within a field, prior to it being planted.

MATERIALS AND METHODS
Soil sampling and assessment: Ten fields of 40 – 50 ha, representing 3 main potato growing regions in South Australia were divided into geographically referenced grids (100 m X 100 m). Composite soil samples consisting of 40 cores (1 cm diameter x 15 cm depth) were collected using an AccuCore soil sampler on a ‘W’ path, inside each grid, 2 months prior to planting and assessed for levels of *C. coccodes* inoculum using real-time PCR (TaqMan®).

Risk zones: Disease risk zones (low = 0 - 10 pg DNA/g soil; medium = 11 - 100pg DNA/g soil; and high = >100 pg DNA/g soil) were identified within each field, based on real time PCR results.

Tuber sampling and assessment: At harvest daughter tubers (cv. Coliban) were collected from 10 replicates in each of the different disease risk zones, washed free of soil and visually assessed for incidence (%) of black dot using a 0 - 4 rating scale, where 0 = no disease, 1 = <5%, 2 = 6 - 25%, 3 = 25 – 50% and 4 = >50% surface area of tuber affected.

RESULTS
Mean *C. coccodes* DNA levels within fields, ranged from 0 - 6964 pg DNA/g soil. Two fields were comprised of multiple risk zones (Fig. 1), while the remainder was comprised of only one risk category, either ‘low, medium or high’.

At harvest, black dot incidence on tubers was shown to have a close link to the different risk zones in which they were grown (Fig. 2).

DISCUSSION
These results confirm that disease risk zones, based on soil DNA tests, provide a reasonable basis for black dot disease prediction in commercial potato fields. Identifying these zones prior to planting will enable growers to avoid high risk regions or implement disease control within the different zones via either husbandry methods (e.g. planting of infected sites in cooler conditions, early harvest) or better targeting chemical treatments to seed/soil through precision farming technology. Implementation of this disease control strategy will lead to environmental and economic benefits to the Australian potato growers through improved tuber quality. Whilst this study shows that levels of *C. coccodes* DNA in soil can provide a good indication of disease risk, further research is required to establish cost effectiveness, soil sampling and validation of these tests on different soil types, moisture regimes and between different potato cultivars.

ACKNOWLEDGEMENTS
We gratefully acknowledge Horticulture Australia Limited for financially supporting this work and the potato growers who allowed research to be conducted on their properties.

The assistance of Russell Burns, Herdina and Ina Dumitrescu (SARDI).

REFERENCES
A BIOASSAY TO SCREEN BIOLOGICAL CONTROL AGENTS AGAINST AERIAL INFECTIONS OF SCLEROTINIA SCLEROTIORUM ON BRASSICA LEAVES

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INTRODUCTION
Cauliflower and other vegetable brassicas are important crops in New Zealand. An important pathogen of these plants, Sclerotinia sclerotiorum, can cause significant rots of maturing plants. In spring, ascospores of the fungus commonly infect petals of seed crops which fall and transfer the infection to the main part of the plant.

The aim of this work was to develop a suitable bioassay to test various biocontrol agents against aerial infection of S. sclerotiorum ascospores. Parameters including wounding versus non-wounding and frozen versus fresh ascospores were tested.

MATERIALS AND METHODS
Collection of New Zealand isolates An isolate of Sclerotinia sclerotiorum was obtained from an infected cauliflower plant at Southbridge. Approximately 1000 fungi and bacteria were isolated from brassica and potato plants and soil samples and tested in vitro for their ability to inhibit S. sclerotiorum mycelial growth.

Production of ascospores Sclerotia of S. sclerotiorum were produced on sterile barley grain at 25°C in Erlenmeyer flasks, cold treated (4°C, dark for 4 weeks), surface sterilised and transferred to deep Petri dishes half filled with moist sterile silica sand, sealed with plastic film, and incubated under UV to produce apothecia. Ascospores were vacuum extracted in sterile 0.05% tween and used and incubated for at least 14 days.

Bioassay experiments Cauliflower leaves of cultivar ‘Devina’ (Seminis, USA) and mustard petals were produced from potted greenhouse grown plants. Preliminary bioassays were conducted in deep Petri dishes containing a layer of moist vermiculite covered by a layer of sterile paper towel. Sterilised cauliflower leaves (one per Petri dish) were prepared: one half non-wounded and the other half wounded with a needle handle. Mustard flower petals provided nutrients and mimicked the natural infection process. Petals were placed on the leaves and inoculated with either fresh or frozen S. sclerotiorum ascospores (~1 x10^6 spores/ml, 10 µl drop) and incubated at 18°C 12D:12N. Lesion diameter was measured. Biocontrol screens used a single wounded leaf, pre-treated with the biocontrol agent (~1 x10^6 spores/ml, air brushed).

RESULTS
Production of ascospores Apothecia and ascospores were produced sufficiently to enable bioassays to be established. Cold treatment and incubation under UV were essential for maximised apothecia production.

Bioassay development Only fungal data is presented. Significant lesion development occurred on non-wounded leaves when fresh ascospores of S. sclerotiorum and mustard petals were used for inoculation (Figure 1). Lesions also occurred when frozen spores and petals were used on non-wounded leaves, but development was delayed. On wounded leaves both fresh and frozen ascospore inoculations resulted in infection and lesion development either with or without mustard petals.

DISCUSSION
This bioassay using wounding and petal inoculation successfully screened some preliminary fungal and bacterial candidates which previously showed activity in vitro against S. sclerotiorum. A detached leaf assay using canola leaves and a petal-mediated infection technique has previously been used to screen biocontrol agents (1). Our bioassay will enable more extensive screening to select candidates for greenhouse and field trials.

ACKNOWLEDGEMENTS
Foundation for Research, Science and Technology.

REFERENCES
FIELD CROP NEMATOLOGY IN SOUTH-EASTERN AUSTRALIA

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INTRODUCTION

In south-eastern Australia, the cereal cyst nematode (CCN), \textit{Heterodera avenae}, and the root lesion nematodes (RLN) \textit{Pratylenchus neglectus} and \textit{P. thornei} are important pathogens of crops of cereal crops (1). Current losses due to these nematodes were recently estimated at $98 million per annum in wheat crops alone (2).

Historically, the Grains Research and Development Corporation (GRDC) with the Victorian Department of Primary Industries (DPIVic) and the South Australian Research and Development Institute (SARDI) had strong capability in nematology research and resistance breeding and this resulted in the development of CCN resistant cereals which greatly reduced widespread losses due to this nematode (1). This research capability was strong until the early 2000s, when changing industry priorities and the success of earlier work resulted in a reduction in research activity.

DPIVic and SARDI, with support from GRDC, are now actively increasing field crop nematology research capacity in an attempt to further reduce crop losses associated with nematodes. The objectives of these new research programs are to evaluate the relative effectiveness of different resistance genes for decreasing nematode population densities, and to determine the relative tolerance to CCN and RLN of recently released cereal cultivars in different environments. This paper reports on initial progress toward these objectives.

MATERIALS AND METHODS

Resistance Cultivars with different resistance levels (as determined from controlled environments screens) will be grown in the field to determine their relative effects on nematode population densities. Field plots (~5 m x 1 m), sown at a site with a low to medium density of the target nematode, will be in a complete randomised block design replicated five times using an approach previously described (3). Following harvest, the nematodes in each plot will be identified and quantified using standard DNA techniques (4).

Tolerance The relative tolerance of recently released cultivars to the target nematode will be determined in the field. In the year prior to tolerance testing, nematode populations will be manipulated using resistant and susceptible crops (Table 1). The test cultivars will then be sown into plots with high and low population densities and the comparative yield measured to determine relative cultivar tolerance. Ideally the cultivars used to manipulate nematode populations in the first year will be similar in terms of water use and nitrogen fixation.

Table 1. Crops used to manipulate nematode population densities in the year prior to field tolerance screening.

<table>
<thead>
<tr>
<th>Nematode</th>
<th>Reaction</th>
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<tbody>
<tr>
<td>\textit{P. thornei}</td>
<td>Field pea</td>
</tr>
<tr>
<td>\textit{P. neglectus}</td>
<td>Triticale</td>
</tr>
<tr>
<td>\textit{H. avenae}</td>
<td>Barley</td>
</tr>
<tr>
<td>\text{\textit{(cv. flagship)}}</td>
<td>\text{\textit{(cv. Gairdner)}}</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

This is a new area of research activity in south-eastern Australia and results from the first field experiments will not be available until after the 2010 growing season.

Resistance Results from the field resistance screening experiments will be compared with the results of controlled environment screening tests for RLN and CCN conducted by Jason Sheedy (DEEDI) at Toowoomba, Queensland and J. Lewis (SARDI) in Adelaide, South Australia. This will enable calibration of screening test results with those obtained from the field in south eastern Australia.

The field resistance screening will also enable the relative effects of different CCN resistance genes (1) and gene combinations on population densities of CCN to be determined. Currently there is limited information on the relative effectiveness of the different CCN resistance genes that are used within modern cultivars in the field.

Tolerance The tolerance screening data generated from the field experiments will be published in annual cereal disease guides to assist growers to select the most tolerant cultivars. Currently there is a paucity of information on field tolerance of cereal cultivars to nematodes.

Once suitable nematode tolerant and intolerant check cultivars are identified, tolerance screening will only need to be conducted at sites with high nematode population densities. This approach will increase the number of lines that can be tested annually and is similar to that used by Thompson et al. (5).

ACKNOWLEDGEMENTS

This research is supported by DPIVic, SARDI and the GRDC.

REFERENCES


6th Australasian Soilborne Diseases Symposium, 2010 55
YIELD LOSS CAUSED BY CROWN ROT IN CEREALS IS RELATED TO PRE-SOWING SOILBORNE PATHOGEN LEVELS AND RAINFALL

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B South Australian Research and Development Institute (SARDI), GPO Box 397, Adelaide, 5001, South Australia
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INTRODUCTION
The advent of qPCR technology has provided plant pathologists with the ability to rapidly identify and quantify DNA levels of important soilborne cereal pathogens before sowing (1). The applicability of this technology is, however, often limited by a lack of relationships between pre-sowing DNA levels of the pathogen and disease severity and subsequent yield loss. To develop such relationships experiments are needed in the field during contrasting seasons, as has been occurring in Victoria for the cereal disease crown rot.

In Victoria, crown rot (caused by Fusarium pseudogibberellatum (Fp) and/or F. culmorum (Fc)) has been shown to be an important disease of wheat that is often worse when September plus October rainfall is below average (2). Growers can reduce crown rot levels using non-host crops (3), but relationships between pre-sowing rot inoculum levels and yield losses are few.

This paper uses data collected from Victorian field trials that studied relationships between pre-sowing inoculum levels and yield losses due to crown rot.

MATERIALS AND METHODS
Plots with the same rotational history were selected from experiments established at Dooen, Victoria to study various aspects of crown rot, including yield loss, and the effects of stubble management and crop rotation (3) on crown rot levels in soil.

These experiments were established using inoculated (Fp or Fc) and un-inoculated seed (3). Rotational and stubble management treatments were applied in the following year. In the final year of these studies Fusarium spp. DNA was quantified using qPCR (3) and plots sown to a cereal that was monitored for disease (white heads and stem browning) and grain yield.

The plots that were included in this analysis were those that had a common rotational history so that yield loss relationships were not confounded by rotational or stubble management effects on soil water and nitrogen levels.

Linear regressions between the densities of Fusarium spp. DNA (Log pg DNA / g soil +1) and grain yields were calculated using GenStat. Rainfall data was obtained from the Bureau of Meteorology.

RESULTS
There were significant negative relationships between pre-sowing crown rot inoculum densities (both Fp and Fc) and cereal yields in seasons when below average rainfall was received (Table 1).

The association between pre-sowing inoculum levels and disease expression was weaker than that for grain yield, even though disease symptoms developed in all trials. Positive relationships with Fp and stem browning were only found in 38% experiments, while for white head development it was found in 14% of experiments. Similarly for Fc positive regressions were only observed for white heads and stem browning in 13% and 25% of trials respectively (data not presented).

| Year | Crop | Yield potential (c)(t/ha) | Slope (m) | Rainfall Sept+Oct (%)
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>2007</td>
<td>Wheat</td>
<td>1.4 -0.19**</td>
<td>-0.07*</td>
<td>31</td>
</tr>
<tr>
<td>2008</td>
<td>Wheat</td>
<td>0.7 -0.07**</td>
<td>0.04</td>
<td>114</td>
</tr>
<tr>
<td>2009</td>
<td>Wheat</td>
<td>2.5 0.04**</td>
<td>-0.10*</td>
<td>114</td>
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<tr>
<td>2009</td>
<td>Wheat</td>
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<td>-0.05*</td>
<td>114</td>
</tr>
<tr>
<td>2007</td>
<td>Durum</td>
<td>0.8 -0.05*</td>
<td>0.01</td>
<td>114</td>
</tr>
<tr>
<td>2009</td>
<td>Durum</td>
<td>2.8 -0.10*</td>
<td>-0.05*</td>
<td>114</td>
</tr>
<tr>
<td>2009</td>
<td>Durum</td>
<td>2.4 -0.05*</td>
<td>-0.07*</td>
<td>114</td>
</tr>
<tr>
<td>2008</td>
<td>Barley</td>
<td>1.5 -0.05**</td>
<td>0.01</td>
<td>114</td>
</tr>
<tr>
<td>2009</td>
<td>Barley</td>
<td>3.2 0.01*</td>
<td>0.01</td>
<td>114</td>
</tr>
</tbody>
</table>

| Year | Crop | Yield potential (c)(t/ha) | Slope (m) | Rainfall Sept+Oct (%)
<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>Wheat</td>
<td>2.0 -0.34 ***</td>
<td>-0.07*</td>
<td>31</td>
</tr>
<tr>
<td>2007</td>
<td>Wheat</td>
<td>1.3 -0.07*</td>
<td>0.05*</td>
<td>114</td>
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<tr>
<td>2009</td>
<td>Wheat</td>
<td>2.7 0.05**</td>
<td>-0.07*</td>
<td>114</td>
</tr>
<tr>
<td>2009</td>
<td>Wheat</td>
<td>2.3 0.01*</td>
<td>0.01</td>
<td>114</td>
</tr>
<tr>
<td>2007</td>
<td>Durum</td>
<td>1.8 -0.42***</td>
<td>0.01</td>
<td>114</td>
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<tr>
<td>2007</td>
<td>Durum</td>
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<td>-0.07*</td>
<td>114</td>
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<tr>
<td>2009</td>
<td>Durum</td>
<td>2.7 -0.07*</td>
<td>-0.05*</td>
<td>114</td>
</tr>
</tbody>
</table>

*** significant at 1%, ** significant at 5%, * significant at 10%, ns not significant, n.s = not significant, 

DISCUSSION
This study supports previous findings that yield losses from crown rot are more severe in seasons with below average September plus October rainfall (2). In addition it establishes relationships between pre-sowing crown rot inoculum levels and grain yield losses in the field.

The findings from these studies will assist growers to determine the risk associated with various pre-sowing crown rot inoculum levels in the field.

ACKNOWLEDGEMENTS
This research is supported by DPIVic, SARDI and the GRDC.

REFERENCES
RHIZOSPHERE BACTERIA ASSOCIATED WITH TWO GRAPEVINE ROOTSTOCKS THAT VARY IN SUSCEPTIBILITY TO CYLINDROCARPON BLACK FOOT DISEASE

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INTRODUCTION
Cylindrocarpon black foot is a major disease of grapevines worldwide. Grapevine rootstocks have been shown to vary in their susceptibility to C. destructans, with Riparia Gloire (RG) having low susceptibility and 101-14 moderate to high susceptibility (1). The population and functionality of rhizosphere bacteria associated with these two rootstock varieties was assessed to determine whether the rootstocks differentially select bacterial populations with the potential to suppress the pathogen.

MATERIALS AND METHODS
Ten rooted cuttings of RG and 101-14 were grown in pots containing 50/50 mix of soil (Wakanui silt loam) and potting mix (80% horticultural bark : 20% pumice). Controls consisted of pots without plants. The pots were arranged in a completely randomised design in a greenhouse. After 8 months growth, rhizosphere soil was collected and culturable bacteria isolated by soil dilution plating onto agar. Total bacterial counts were assessed on nutrient agar (NA), fluorescent pseudomonads on Kings B agar (KB) under UV light and spore forming bacteria on NA after heat treating soil dilutions at 80ºC for 10 min. From these plates, 40 bacteria were randomly selected from each treatment and subcultured into pure culture.

Functionality testing The ability of the bacteria to inhibit C. destructans growth was assessed by a dual-culture assay. Potato dextrose agar plates were inoculated centrally with a C. destructans plug and also with 10 µl of bacterial broth at four equidistant points around the perimeter of the plate. Zones of inhibition were assessed after 10 days growth at 20ºC. Control plates were set up using sterile water. Siderophore production, β-glucanase and protease activity were assessed (2). There were six replicate plates per treatment.

RESULTS AND DISCUSSION
There was no significant difference in the numbers of total or spore forming bacteria recovered from the rhizosphere of the rootstocks compared with the control (Table 1). Fluorescent pseudomonads were not isolated from the control soil, and their numbers did not differ significantly between the two rootstocks (Table 1).

Table 1. Total bacteria, spore forming and fluorescent pseudomonad counts (log_{10} CFU/g soil) isolated from the rhizosphere soil of two grapevine rootstocks.

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Log_{10} Total CFU/g dry soil</th>
<th>Log_{10} Spore former CFU/g dry soil</th>
<th>Log_{10} Fluorescent pseudomonad CFU/g dry soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.3</td>
<td>4.8</td>
<td>0</td>
</tr>
<tr>
<td>101-14</td>
<td>5.5</td>
<td>4.6</td>
<td>3.0</td>
</tr>
<tr>
<td>RG</td>
<td>5.5</td>
<td>4.7</td>
<td>1.2</td>
</tr>
<tr>
<td>LSD</td>
<td>LSD</td>
<td>LSD</td>
<td>LSD</td>
</tr>
</tbody>
</table>

There was no significant difference (χ² test; P = 0.545) in the proportion of bacteria inhabiting C. destructans between 101-14, RG and the control (Fig 1). The degree of siderophore activity was significantly different between 101-14, RG and the control (χ² test; P = 0.000). The proportion of bacteria producing β-glucanase was significantly different between 101-14, RG and the control, (χ² test; P = 0.000). Similarly the proportion of bacteria producing protease was significantly different between 101-14, RG and the control (χ² test; P = 0.001).

A higher proportion of the bacteria closely associated with plant roots (rhizosphere bacteria) had high functional activity when compared to bulk control soil. A larger proportion of bacteria with high siderophore and β-glucanase activity were isolated from the rhizosphere of RG, which has low susceptibility to C. destructans, compared with the susceptible rootstock 101-14. The most resistant rootstock RG appeared to select for a higher proportion of bacteria with functional traits which have been correlated to disease suppression ability.

ACKNOWLEDGEMENTS
New Zealand Winegrowers for funding.

REFERENCES

Figure 1. Proportion of bacteria isolated from the rhizosphere of 101-14 and RG rootstocks with the following functional traits: (a) C. destructans inhibition, (b) siderophore production, (c) β-glucanase activity, (d) protease activity. - none, + low, ++ moderate-high activity.
DOES ADDITION OF THE ELEMENT SILICON AFFECT THE INFECTION PROCESS OF FUSARIUM OXYSPORUM F. SP. CUBENSE ON BANANA?

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INTRODUCTION

The relentless spread of fusarium wilt (Fusarium oxysporum f. sp. cubense (Foc)) through banana plantations across the globe has generated the necessity for innovative control methods. Breeding for resistance remains the ultimate goal in combating fusarium wilt, but an easily deployable, resistant commercial cultivar remains elusive.

Novel control methods currently being explored include beneficial endophytic bacteria and fungi, new field management techniques, bio-control fungi targeting Fusarium and various soil amendments. The focus of this research project is the application of silicon as a soil amendment. Silicon application has been demonstrated to enhance the defence response of plants against various pathogens and various negative abiotic factors, such as heat stress and lodging\(^1\).

The aims of this project are twofold: 1) to determine what effect silicon application is having on fusarium pathogenesis in banana; and 2) to determine whether silicon reduces Foc symptoms in inoculated Cavendish banana plants.

MATERIALS AND METHODS

Cavendish ‘Williams’ banana plants were tissue cultured, deflasked and grown in a glasshouse for three months. Plants were subsequently inoculated with Foc infested millet grains. External and internal disease symptoms were evaluated at 4 weeks post inoculation.

Roots were harvested at 3 days post inoculation and were fixed in 2.5% gluteraldehyde in phosphate buffer with 0.7% caffeine and then processed for transmission electron microscopy (TEM). Thin sections (80nm) were cut and viewed on a JEOI1010 transmission electron microscope. Internal cellular responses, such as accumulation of phenolics, wall apposition and hyphal survival were investigated.

RESULTS

Disease assessment at 4 weeks showed the beginnings of wilt symptoms in all inoculated plants, including splitting at the base of pseudostems, deformed leaf initials, and chlorotic leaves beginning with the oldest. Leaf yellowing was rated on a scale from 0% (no yellowing) to 100% (where all leaves were totally chlorotic). All uninoculated plants showed negligible amounts of yellowing. Potassium silicate application showed a significant decrease in leaf yellowing compared to the untreated control (Fig 1).

Hyphae observed in silicon treated samples at 3 days post inoculation were mostly external to the root, highly vacuolated, swollen and had alterations in their cell wall (Fig 2). Whereas in the untreated controls, hyphae were unchanged and had begun colonising intercellular and intracellular spaces within the banana root.

DISCUSSION

Results showing a decrease in symptoms indicate that silicon is either inhibiting Foc pathogenesis or enhancing plant tolerance to the disease. The mechanism by which silicon works has yet to be elucidated.

Imaging results suggest that silicon is inducing a change in the defence response of banana. Increased hyphal vacuolation and swelling coupled with diminished cell walls is often indicative of chitinase activity. Research in this area is ongoing.

ACKNOWLEDGEMENTS

Many thanks to the Australian Banana Grower’s council and Horticultural Australia for funding this project.

REFERENCES

INTRODUCTION

Trichoderma species are fungi that commonly occur in nearly all agricultural soils and selected strains can suppress plant diseases. However, their level of efficacy and their reliability as a biocontrol agent depends upon their ability to tolerate adverse environmental conditions.

Trehalose is a non-reducing disaccharide, osmoprotectant and a common energy reserve in fungi. It is stable under hot and acidic conditions and can protect biological structures during freezing, desiccation or excess heating.

Prolonged or intensive exposure of a cell or fungal spore to heat or chemical stress can temporarily increase trehalose levels but these return to normal on removal of the stress (1). However, osmoadapted cells accumulate trehalose and glycine betaine (GB) intracellularly and show a higher tolerance to desiccation than non-adapted cells (2). In the present study, Trichoderma atroviride (LU132) spores with increased trehalose levels were compared to spores with normal levels for their tolerance to various environmental stress factors.

MATERIALS AND METHODS

T. atroviride (LU132) spores were produced from cultures grown on potato dextrose agar (PDA) amended with 1mM Trehalose (TS) and compared with spores grown on unamended PDA. The accumulated trehalose content in the spores was estimated by digesting trehalose to glucose with the enzyme trehalase and measuring absorbance at 340nm. Spores were stored at 4°C in desiccators.

Germination percentage of spores 500 μl spore suspensions (2×10^7 spores/ml) of LU132 and LU132-TS (trehalose supplemented) were added to 500μl of Potato Dextrose (PD) broth. Tubes were incubated overnight for 18 hours at 22°C rotating at 35pm/min. Germinated spores were counted and expressed as percent germination.

Effect of temperature and pH on colony growth rate

Normal PDA plates and plates with pH values ranging from 2-6 were inoculated with 15μl aliquots of the spore suspensions (at 1×10^7 spores/ml) of LU132 and LU132-TS. They were incubated at three different temperatures (15°C, 25°C, 35°C) and growth rate/day was calculated.

Stress tolerance

500μl aliquots of LU132 and LU132-TS spore suspensions (at 2×10^7 spores/ml) were placed into several Eppendorf tubes. Replicate tubes were subjected to different temperature/exposure time treatments (see Figure 1). Each tube was then mixed with 500 μl of PD broth and the germination was calculated as above.

UV-B treatment

Petri plates with 7ml of spore suspensions of LU132 and LU132-TS (at 4×10^7 spores/ml) were exposed to UV-B fluorescent lamps (TL20W/12RS Philips). The lamps were covered with a 0.13mm-thick cellulose diacetate film to block radiation below 290nm. During irradiation the plates were kept at a distance of 21cm from the lamp and gently agitated. Control plates were covered with aluminium foil and samples collected after 1, 2, 3 and 4h exposure. Percent spore germination was calculated.

RESULTS

T. atroviride spores harvested from cultures grown on 1mM amended PDA plates contained 49.11ng/mg trehalose compared to 43.11ng/mg from unamended PDA plates, a 15.3% increase. Their germination was significantly higher (Table 1) and their tolerance to temperature stress was significantly increased (Figure 1). No statistical difference was observed between growth at different temperatures and pH values.

Table 1. Percent spore germination of T. atroviride spores harvested from cultures grown on 1mM amended PDA plates.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage</th>
<th>LSD</th>
<th>F pr</th>
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<tbody>
<tr>
<td>LU132</td>
<td>69.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LU132-TS</td>
<td>77.29</td>
<td>4.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

T. atroviride spores with increased trehalose tolerated UV-B radiation significantly better than unamended spores, with 75% and 65% germination respectively.

DISCUSSION

This study has revealed that the incorporation of trehalose into T. atroviride spores can significantly improve their tolerance to various biological stress factors including temperature and UV. Bonaterra et al. (2) also showed that spores with higher trehalose content had better stress tolerance especially to desiccation. Several authors have shown that accumulation of trehalose in fungal hyphae can also increase their tolerance to lower temperatures (3). Improved tolerance of spores to stress should ultimately increase the shelf-life of potential biocontrol agents.

ACKNOWLEDGEMENTS

Foundation for Research, Science and Technology.

REFERENCES

GROWTH PROMOTION AND BIOLOGICAL CONTROL OF RHIZOCTONIA SOLANI IN OILSEED RAPE USING BENEFICIAL BACTERIAL ISOLATES

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INTRODUCTION
Oilseed rape (Brassica napus) is now grown in New Zealand as a biofuel crop. However, many Brassica crops in New Zealand can suffer from damping-off and root-rot diseases (1) caused predominantly by Rhizoctonia solani and Sclerotinia sclerotiorum. The application of beneficial bacteria to crop seeds has previously been used successfully to improve seedling establishment (2). The present work is part of a project in which we are evaluating the ability of bio-active microbes to enhance oilseed rape seedling establishment, plant growth and oil quality.

MATERIALS AND METHODS
Bacterial isolates Five bacterial isolates from Brassica seeds (BSB1-5) were selected from an in vitro mycelial inhibition bioassay for their ability to produce strong inhibition zones against a highly virulent isolate of R. solani (R73-13b) from the Centre culture collection.

RESULTS
Glasshouse experiments Two sets of pot experiments were conducted during summer 2009 (February-March) using a randomised block design (3 blocks with 5 replications of each treatment/block). Mean temperature in the glasshouse was 20±2°C during the experimental period. Bacterial isolates were grown on Luria broth to yield 10^8 colony forming units (CFU)/mL and were tested for their ability to promote plant growth in pathogen free potting-mix (Brassica napus var Ability) when challenged by artificially inoculated R. solani (Experiment 2). The potting-mix inoculated with R. solani (0.25% w/w mycelial inoculum multiplied on wheat bran) prior to seed (10 seeds per pot) sowing. The bacterial isolates were applied as a drench @ 4 mL/pot to give 10^6 CFU/g of potting-mix. All experiments were watered regularly to avoid water-stress. Seedling emergence was counted 3 weeks after sowing. Assessment of disease (wire-stem symptoms), shoot and root dry matter (DM) was undertaken at 7 weeks after sowing.

RESULTS
Experiment 1 In pathogen-free conditions, seedling emergence from the bacterial treatments did not differ from the potting-mix control (Table 1) although there were significant differences among bacterial treatments.

Table 1. Effect of five bacterial isolates on oilseed rape seedling emergence, shoot and root dry matter when grown in pathogen free potting-mix.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Emergence (%)</th>
<th>Shoot DM (g)</th>
<th>Root DM (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potting-mix</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>92 ab</td>
<td>3.65 a</td>
<td>1.75 a</td>
</tr>
<tr>
<td>BSB1</td>
<td>88 a</td>
<td>3.62 a</td>
<td>1.59 a</td>
</tr>
<tr>
<td>BSB2</td>
<td>96 b</td>
<td>3.89 ab</td>
<td>1.79 a</td>
</tr>
<tr>
<td>BSB3</td>
<td>88 a</td>
<td>4.18 b</td>
<td>2.27 b</td>
</tr>
<tr>
<td>BSB4</td>
<td>91 ab</td>
<td>3.54 a</td>
<td>1.69 a</td>
</tr>
<tr>
<td>BSB5</td>
<td>85 a</td>
<td>3.71 a</td>
<td>1.66 a</td>
</tr>
<tr>
<td>LSD (P=0.05)</td>
<td>7</td>
<td>0.352</td>
<td>0.441</td>
</tr>
</tbody>
</table>

Note: Values followed by different letters in each column are significantly different.

DISCUSSION
Beneficial microorganisms applied to seed or roots in an appropriate formulation may promote plant growth or provide disease control through a variety of mechanisms (2). The bacterial isolates used in this study were obtained from New Zealand-grown Brassica seed lots. Four of the bacteria were able to reduce diseased plants to some extent (by 20-32%) with BSB3 also promoting plant growth in the absence of the pathogen. This isolate was identified as a Bacillus sp. Further studies will be conducted with this isolate to optimize inoculum rate and application method and evaluate bioactivity under field conditions.

REFERENCES
HISTOPATHOLOGICAL INVESTIGATION OF FUSARIUM CROWN ROT IN WHEAT

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BDEEDI, Primary Industries and Fisheries, Leslie Research Centre, Toowoomba, 4350, QLD
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INTRODUCTION
Crown rot of wheat, caused by Fusarium pseudogibbsiae (Fp), is a serious stubble-borne disease threat across the Australian wheat belt. Partial resistance has been identified in a small number of wheat lines, such as 2-49 and Sunco, but the mechanisms of resistance shown by these lines have not been identified. Partial resistance can be expressed in either the seedling or adult stage, depending on the genotype. Extensive seedling trial comparisons between susceptible and partially resistant host genotypes suggest a significantly slower spread of the fungus in the younger tissues of resistant individuals (1, 2). This study is the first to microscopically examine Fp infection and hyphal growth patterns in seedling and adult plants from partially resistant and susceptible wheat lines.

MATERIALS AND METHODS
 inoculation Two week old seedlings were inoculated with 6 μL of a 10^6 conidia per mL suspension (3). Seedling tissues were collected at various times after inoculation, ranging from 3 to 21 days. Adult plants were collected from an infected experimental field plot at Wellcamp outside Toowoomba, Qld. Adult tissues were collected at 10, 16 and 22 weeks after planting. While various wheat lines were assessed, the partially resistant genotype 2-49 and the susceptible lines were included in all experiments.

Microscopy Fixation and clearing of tissues was performed as previously described (4). Differential staining employed safranin and solophenyl flavine dyes and tissues were examined using a Nikon Eclipse fluorescence microscope under a UV-2A filter.

RESULTS AND DISCUSSION
Seedlings Microscopic examination of Fp growth in seedlings revealed that hyphae proliferated both on the surface of the leaf sheath and internally; mostly in the epidermal tissues. Surface growth was more extensive on less exposed surfaces, such as those between leaf sheaths.

Initial tissue infection predominantly occurred through stomata (Fig. 1), with trichomes and wounds providing additional entry points. Both intra- and inter-cellular growth were observed in leaf sheath tissue, with appressoria-like structures appearing to facilitate cell wall crossing events. After initial internal growth, hyphae grew prolifically out of the stomata. As the leaf sheath began to senesce, hyphae exterior to the stomata produced conidia. Fp hyphae typically grew within the leaf sheath tissue between vascular bundles, which were infrequently colonised. Hyphae which grew in cells adjacent to vascular bundles showed an altered, thickened appearance (Fig. 2).

Figure 1. Unstained, cleared leaf sheath. Lesions originating at stomata (+) and trichomes (+). Fluorescence microscopy revealed hyphae in the stomatal apertures.

Figure 2. Leaf sheath stained with safranin and solophenyl flavine. Thickened hyphae (th) and appressoria-like structures (a), present within the adaxial tissue above a vascular bundle.

Adult Plants Initial infection of expanded tillers occurred primarily in the epidermis and outer cortex of the culm base, followed by the pith. Penetration from colonised leaf sheaths through the stem epidermis was also observed. Upward spread in each internode appeared to be most rapid in the pith cells and pith cavity (where present). During severe infection, hyphae were observed in all tissue types, including both xylem and phloem.

A clear understanding of Fp growth patterns in cereals will greatly assist both germplasm screening methods and crop management strategies aimed at disease reduction. Previous studies, based on qPCR and visible disease scoring, indicate that Fp shows significant differences in the rate of hyphal spread between partially resistant and susceptible genotypes (2). However, no constitutive or induced structural variations, which might explain these differences, have yet been detected in these ongoing studies.

REFERENCES
INTRODUCTION
Ginger is a small but economically important crop in Australia, worth AUD 80 million pa. Since 2007, ginger growers in Australia have faced severe yield losses, due to Pythium soft rot disease. *P. myriotylum* Drechs. has been identified causing this rot (1); however other *Pythium* spp. have also been implicated (2). In this study we have investigated the role of several *Pythium* species in this soft rot disease in ginger fields in Australia.

MATERIALS AND METHODS

Morphological identification: *Pythium* spp. isolates were obtained from diseased ginger rhizomes, or from diseased oat seedlings in a ginger follow-up crop, from ginger farms in the SE Queensland. Morphological characteristics were examined including oogonia, oospores, antheridia, zoospores, sporangia and appressoria. These fruiting structures were induced by floating a small autoclaved sorghum leaf alongside a small square of *Pythium* on PDA (potato dextrose agar) in a shallow Petri dish containing sterile soil extract (3); these plates were then kept at 27°C in the dark for 2-3 days. In addition, cardinal temperatures were determined for each isolate by growth on PDA over a range from 25°C to 40°C.

Genetic identification: In order to support morphological identification, DNA sequencing of the ITS region including, *P. myriotylum* and *P. zingiberis* were determined for each isolate by growth on PDA over a range from 25°C to 40°C. Isolates from ginger conformed to *P. myriotylum* and *P. zingiberis* which cause crop diseases. The distinction between these two species at a genetic, taxonomic and pathogenetic level and to determine which is the main destructive agent on ginger in Australia.

ACKNOWLEDGEMENT
We would like to thank Dr Marcelle Stirling for providing isolates of *Pythium* spp; also Ms Regina Sintrajaya, Mr David Amour, Mr Sam Fraser Smith and Mr Kevan W. Jones for technical assistance.

REFERENCES

PYTHIUM SPP. ON GINGER (ZINGIBER OFFICINALE ROSCOE) IN AUSTRALIA

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RESULTS AND DISCUSSION

Morphological identification: Different *Pythium* spp. were identified based on keys of Van der Plaats-Niterink (5). One of the isolates from oats was identified as *P. irregulare*; it formed smooth-wall and intercalary oogonia sitting from 15 to 30 µm dia and aplerotic oospores ranging from 15 to 20 µm dia; a maximum of two antheridia per oogonium; no sporangia nor zoospores; many global hyphal swellings at terminal and intercalary positions and an optimum growth in culture at 30°C with no growth at 35°C. Also from oats, isolates of *P. spinosum* were identified, these produced: ornamented oogonia ranging from 15 to 20 µm; aplerotic oospores (oospores filling oogonia) measuring from 15 to 20 µm; one antheridium per oogonium; no sporangia or zoospores; optimum growth on media at 25°C with no growth at 35°C. Isolates from ginger conformed generally with the descriptions of *P. myriotylum*: they formed appressoria always in a cluster; aplerotic oospores up to 37 µm dia and up to six per oogonium; filamentous and slightly inflated sporangia; zoospores from 5 to 15 µm dia; optimum growth on media varying from 30 to 37°C with no growth at 40°C for most of the isolates. However the oogonia were in some cases larger than expected for *P. myriotylum*, being up to 40 µm dia. This difference in size could be due to conditions used to induce oogonia (soil extract versus water) or due to the presence of another *Pythium* species, *P. zingiberis* (6).

Genetic identification: ITS sequence analyses enabled confirmation of four *Pythium* species (*P. irregulare, P. spinosum, P. myriotylum* and *P. zingiberis*) which completely matched the deposited sequences in GenBank using BLAST. The latter two species differ by one base pair in the ITS region (8).

Pathogenicity tests: At 10 DAI, symptoms of leaf sheath collar discolouration and yellowing at lower leaves appeared on ginger shoots infected with the isolates identified as *P. myriotylum* and *P. zingiberis*. By 30 DAI these plants showed severe disease symptoms. In comparison, plants inoculated with the isolate identified as *P. spinosum* did not develop any symptoms on living ginger plants; *P. irregulare* isolates were not tested at this stage for pathogenicity on plants nor for growth on excised ginger pieces. All the isolates which were tested on the excised ginger pieces grew on the rhizomes but at different rates. The *P. spinosum* isolate was amongst the slowest whereas one isolate that diverged slightly from both *P. myriotylum* and *P. zingiberis* in the sequence analysis, showed particularly aggressive growth on the excised ginger pieces.

The distinction between *P. myriotylum* and *P. zingiberis* (7) needs to be resolved. Work is required to differentiate these two species at a genetic, taxonomic and pathogenetic level and to determine which is the main destructive agent on ginger in Australia.

6th Australasian Soilborne Diseases Symposium, 2010
INTRODUCTION

Nematode community analysis is often used to make inferences about the condition of the soil food web. In this study, we compared nematode assemblages in soils under crop or pasture at different depths in the profile.

MATERIALS AND METHODS

In January 2010, three replicate soil samples from depths of 0-10, 20-30 and 40-50 cm were collected from adjacent fields that had been cropped to wheat, sorghum and chickpea for many years or were under permanent pasture. Soil (200 g) was placed on Whitehead trays for 2 days and nematodes were recovered on a 38 um sieve. After enumeration, nematodes were fixed in 4% formaldehyde and about 150 specimens per sample were randomly selected and identified. Each nematode was assigned to a trophic group, and the faunal profiles were produced as described by Ferris et al. (1).

RESULTS AND DISCUSSION

A total of 35 and 29 taxa were found in pasture and cropped soils, respectively. In general, taxonomic richness was higher in pasture than crop (P<0.01, data not shown). Total nematode population densities at the two sites were similar (Table 1) but there were more nematodes in pasture than cropped soil (12.6 v. 5.5 nematodes/g soil). The predominant plant-parasitic nematodes (Pp) were Pratylenchus and Merlinius in cropped soils and Pratylenchus and Criconemella in pastures.

Table 1. Effects of site, land use and depth on nematodes in various trophic groups (nematodes/g soil).

<table>
<thead>
<tr>
<th></th>
<th>Pp</th>
<th>Ba</th>
<th>Fu</th>
<th>Om</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jimbour</td>
<td>1.6a</td>
<td>1.8a</td>
<td>3.6a</td>
<td>1.0a</td>
<td>0.25a</td>
</tr>
<tr>
<td>Kingsthorpe</td>
<td>2.8a</td>
<td>2.5a</td>
<td>3.5a</td>
<td>0.8b</td>
<td>0.18b</td>
</tr>
<tr>
<td>Crop (C)</td>
<td>1.4b</td>
<td>1.7b</td>
<td>1.8b</td>
<td>0.5b</td>
<td>0.15b</td>
</tr>
<tr>
<td>Pasture (P)</td>
<td>3.0a</td>
<td>2.6a</td>
<td>5.3a</td>
<td>1.3a</td>
<td>0.28a</td>
</tr>
<tr>
<td>0-10 cm</td>
<td>0.6b</td>
<td>3.9a</td>
<td>5.5a</td>
<td>1.4a</td>
<td>0.41a</td>
</tr>
<tr>
<td>20-30 cm</td>
<td>3.3a</td>
<td>1.7b</td>
<td>2.9b</td>
<td>0.8b</td>
<td>0.17b</td>
</tr>
<tr>
<td>40-50 cm</td>
<td>2.6a</td>
<td>0.8b</td>
<td>2.3b</td>
<td>0.5c</td>
<td>0.05b</td>
</tr>
<tr>
<td>Site* Land use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jimbour C</td>
<td>1.2a</td>
<td>2.0a</td>
<td>2.6bc</td>
<td>0.8b</td>
<td>0.28a</td>
</tr>
<tr>
<td>K‘thorpe C</td>
<td>1.5a</td>
<td>1.4b</td>
<td>1.0c</td>
<td>0.1c</td>
<td>0.02b</td>
</tr>
<tr>
<td>Jimbour P</td>
<td>2.0a</td>
<td>1.6ab</td>
<td>4.6ab</td>
<td>1.3a</td>
<td>0.22a</td>
</tr>
<tr>
<td>K‘thorpe P</td>
<td>4.0a</td>
<td>3.6a</td>
<td>6.1a</td>
<td>1.4a</td>
<td>0.34a</td>
</tr>
</tbody>
</table>

Site, land use and depth effects are significant (P<0.05) if numbers within a column are followed by different letters.

When free-living nematodes were separated into trophic groups, there were sometimes interactions between site and land use, but there were generally more bacterivores (Ba), fungivores (Fu), omnivores (Om) and carnivores (Ca) in pasture than cropped soil. Depth effects were consistent across sites, with numbers of plant parasites increasing and numbers of free-living nematodes decreasing with depth (Table 1). Since root biomass declined with depth, top-down predation appeared to be regulating populations of plant parasites whereas the free-living nematode community was being regulated by resource availability.

The enrichment index (EI) showed that the proportion of bacterial-feeding enrichment opportunists in the community was not affected by land use or depth. However, the structure index (SI) was higher under pasture than in cropped soil (indicating a higher proportion of omnivores and predators) while the channel index (CI) increased with depth, indicating that the relative importance of bacterial decomposition channels in the detritus food web declined with depth (Table 2).

Table 2. Effects of site, land use and depth on indices generated from nematode community analysis.

<table>
<thead>
<tr>
<th></th>
<th>EL</th>
<th>SI</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jimbour</td>
<td>60.4a</td>
<td>78.6a</td>
<td>48.1a</td>
</tr>
<tr>
<td>Kingsthorpe</td>
<td>56.8a</td>
<td>65.4b</td>
<td>49.9a</td>
</tr>
<tr>
<td>Land use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crop</td>
<td>55.9a</td>
<td>63.0b</td>
<td>52.7a</td>
</tr>
<tr>
<td>Pasture</td>
<td>61.3a</td>
<td>81.0a</td>
<td>45.3a</td>
</tr>
<tr>
<td>Depth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10 cm</td>
<td>61.8a</td>
<td>66.3a</td>
<td>38.3b</td>
</tr>
<tr>
<td>20-30 cm</td>
<td>57.6a</td>
<td>75.1a</td>
<td>47.6ab</td>
</tr>
<tr>
<td>40-50 cm</td>
<td>56.5a</td>
<td>74.7a</td>
<td>61.2a</td>
</tr>
</tbody>
</table>

Site, land use and depth effects are significant (P<0.05) if numbers within a column are followed by different letters.

Previous studies have shown that crop management practices and pasture species composition influence nematode communities in cropped and pasture soils from northern Australia (2, 3, 4). Our comparison of adjacent cropped and pasture soils showed that pasture soils had more nematodes, greater diversity within the nematode community, more omnivorous nematodes and greater numbers of predatory nematodes. Although only two sites were studied, we suggest that the biological status of soils under pasture is much better than cropped soils.

ACKNOWLEDGEMENTS

We thank Jenny Cobon for her support and DEEDI for providing laboratory facilities.

REFERENCES

BIOLOGICAL FACTORS INFLUENCE NEMATODE DISTRIBUTION IN VERTOSOLS FROM THE NORTHERN GRAIN-GROWING REGION

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INTRODUCTION
Although root-lesion nematode (Pratylenchus thornei) is a major pathogen of wheat in northern NSW and Queensland, previous research has shown that soils from depths of 0-15 cm are suppressive to the nematodes (1). In a series of pot experiments over 4 years, populations of P. thornei increased only four times when wheat was grown in untreated soil compared with a 5-21 fold increase when soils were sterilised by heat, gamma irradiation or fumigation. The addition of 10% field soil to sterilised soil reduced nematode multiplication rates by 75-85%, while populations of P. thornei did not increase above initial inoculum densities in soil from some fields. In this study, an assay with Radopholus similis, a nematode that does not occur in grain-growing soils, was used to compare the suppressiveness of soils from different depths in the profile. Preliminary observations on organisms possibly associated with suppression are also reported.

MATERIALS AND METHODS
Three replicate samples of cropped and pasture soils from 3 sites on the Darling Downs were collected in January 2010 from 2 depths (0-10 and 40-50 cm) and half the soil was partially sterilised by heat (65°C for 2 hours). The suppressiveness of soils at 0-10cm depth that were disturbed during the sampling process was compared to undisturbed soil (collected as cores) by adding 1,600 R. similis to heated and unheated soil and extracting nematodes 8 days later. The same assay (with 4,000 R. similis) was also used to assess the suppressiveness of cropped and pasture soils from Kingsthorpe and Jimbour at depths of 0-10 and 40-50 cm. Predatory nematodes in each sample were identified and nematode-trapping fungi were isolated by adding 1 g and 0.1 g soil, respectively, to sprinkle-plate and dilution-plates.

RESULTS
In the first bioassay, similar numbers of R. similis were recovered from each site and from disturbed and undisturbed soil. However, fewer nematodes were recovered from unheated than heat-sterilised soil (Table 1).

Table 1. Main effects in an assay where Radopholus similis was added to cropped soils from 0-10 cm depth (3 sites × 2 vegetation types × 2 depths × ± heat × 3 replicates).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Number of R. similis recovered from 200 ml soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site**</td>
<td>T’hompe  Jimbour  Jandowae</td>
</tr>
<tr>
<td>Heat**</td>
<td>Heated  Unheated</td>
</tr>
<tr>
<td>Disturbance**</td>
<td>Disturbed  Undisturbed</td>
</tr>
</tbody>
</table>

In each row, numbers followed by the same letter are not significantly different (P<0.05). ANOVA: *P<0.05, **P<0.01.

In the second bioassay, 37-49% fewer R. similis were recovered from unheated than heated soil in all treatments except the 0-10 cm pasture soil, where the reduction was 71-84%. Site did not affect the number of R. similis recovered, but there were significant effects of heat, vegetation type and depth. There was also an interaction between vegetation type and depth (Table 2).

Table 2. Main effects and significant interactions in an assay where Radopholus similis was added to cropped and pasture soils (2 sites × 2 vegetation types × 2 depths × ± heat × 3 replicates).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Number of R. similis recovered from 100 g soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site**</td>
<td>Kingsthorpe  Jimbour</td>
</tr>
<tr>
<td>Heat**</td>
<td>Heated  Unheated</td>
</tr>
<tr>
<td>Vegetation type*</td>
<td>Crop  Pasture</td>
</tr>
<tr>
<td>Depth**</td>
<td>0-10 cm  40-50 cm</td>
</tr>
<tr>
<td>Vegetation × Depth**</td>
<td>Crop  Pasture</td>
</tr>
<tr>
<td>0-10 cm  40-50 cm</td>
<td>1030 a  1045 a</td>
</tr>
</tbody>
</table>

In each row, numbers followed by the same letter are not significantly different (P<0.05). ANOVA: *, P<0.05, **, P<0.01.

The proportion of predators in the nematode community was not affected by vegetation type, but was greatest in surface soil and decreased with depth. Iotolynchus, Mylchnochus and Tripyla were the most common predators, but Actinolaimus, Nygolaimus and some Discolaimidae were also observed.

Nematophagous fungi were observed infrequently on sprinkle plates and were not recovered from dilution plates. The species present were Arthrobotrys conoides, A. oligospora, Gamysella gephyropaga and Stylopage sp.

DISCUSSION
Since fewer R. similis were recovered from unheated than heated soil in both our bioassays, these results support previous observations (1) that soils from the Darling Downs contain organisms that suppress plant-parasitic nematodes.

In our second bioassay, there was an interaction between vegetation type and depth, with fewer R. similis recovered from the upper layer of the pasture soils than from either the cropped soil or the pasture soil at depth. This indicates that the pasture soils were much more suppressive to nematodes in the 0-10 cm zone than further down the profile.

Several parasites and predators of nematodes were recovered from the soils, but further work will be required to determine whether they are associated with the suppressiveness observed.

ACKNOWLEDGEMENTS
We thank Jenny Cobon for her support and DEEDI for providing laboratory facilities.

REFERENCES
MONITORING ROOT AND LEAF SALICYLIC ACID TO OPTIMISE INDUCTION OF SYSTEMIC ACQUIRED RESISTANCE IN BROCCOLI

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INTRODUCTION
The phytohormone, salicylic acid (SA), is required for a number of physiological processes within plants but primarily it is an important signalling molecule in plant defence, at both cellular and tissue levels but also systemically (1). SA is implicated as a signal in defence against pathogens via systemic acquired resistance (SAR), a mechanism of induced defence that confers long-lasting protection against a broad spectrum of microorganisms (2). We are investigating SA-induced SAR in broccoli following inoculation with Plasmiodiophora brassicae. Biochemical methods have been developed to measure SAR induction in broccoli for the first time. Extraction from broccoli tissue and High Performance Liquid Chromatography (HPLC) analysis has been optimised to quantify SA levels post induction.

MATERIALS AND METHODS
Trays or pots containing 10 to 14 day old broccoli seedlings were dipped for 15 minutes in 1 mM SA once or three times (once daily for 3 days). Root and leaf sample pairs were collected from the same plant 12, 24, 48, 72 and 216 hours post treatment. SA was extracted from the plants using a modified method (3). Briefly, plant material was ground in liquid nitrogen and SA was extracted twice with methanol (90% and 100%) and an ethylacetate/trichloroacetic acid (5%) 1:1 (v/v) mix and dried in a speed vac. SA was resuspended in a mobile phase (0.2 M KAc/0.5 mM EDTA [pH 5]) and passed through a 250mm x 0.5µm C18 HPLC column. Concentrations of SA were determined by reverse phase (RP)-HPLC. In order to calculate the concentration of SA (µM) in the roots and shoots of treated broccoli a set of standards (0.5 µM, 1 µM, 2 µM, 5 µM, 10 µM, 20 µM, 50 µM and 100 µM) were analysed by RP-HPLC and a standard curve was produced.

Experiments are currently being conducted using real-time quantitative RT-PCR (RT-qPCR) to monitor changes in the expression of 3 defence related genes PR-1, PR-2 and chitinase. Actin8 is used as the reference gene. Total RNA will be isolated from root and leaf sample pairs collected 24 hours post treatment with SA (0.1 to 1 mM), treated with DNase 1 and then reverse transcribed into cDNA. RT-qPCR will be conducted and changes in gene expression analysed using the comparative Ct method (ΔΔCt) for calculating the relative fold change of genes.

RESULTS AND DISCUSSION
SA levels rose rapidly in root and shoot tissue peaking between 48 and 72 hours after treatment. The maximum concentration of SA in triple-dipped root tissue was calculated to be more than twice that of single-dipped roots (Figure 1).

Root SA decreased rapidly in triple-dipped plants but not in single-dipped plants such that 216 hours after treatment there was no significant difference (p=0.05) in the relative concentration of SA in triple or single-dipped roots. This may be important information in developing an SA treatment plan for young broccoli seedlings as multiple dips are difficult to apply commercially and the extra SA can have a phytotoxic effect on seedlings.

Treatment with 1 mM SA caused symptoms of phytotoxicity, including leaf burn and stunting in the plants. Lower concentrations of 0.1 mM, 0.25 mM and 0.5 mM are being tested to determine their potential to stimulate SAR and control clubroot disease without causing phytotoxic symptoms in treated plants.

Molecular techniques such as RT-qPCR are currently being utilised to determine the effect of SA on the defence responses of broccoli, specifically SAR. This technique in conjunction with RP-HPLC and microscopy will further enhance our understanding of the relationship between broccoli and P. brassicae.

ACKNOWLEDGEMENTS
This work has been funded by DPI Victoria and Horticulture Australia Limited (HAL) using the vegetable levy and matched funds from the Australian Government. We thank Dr. Xavier Conlan, Deakin University, Geelong, for providing assistance in the SA analysis. D. Lovelock is funded by a DPI-Deakin University Post-Graduate Scholarship.

REFERENCES

Figure 1. Concentration of SA (µM) within roots in both single and triple-dip treated plants obtained from HPLC analysis at 0, 24, 48, 72 and 216 h after treatment. 0 h is the untreated control. Letters indicate a significant difference (p = 0.05). Error bars represent the standard error.
PROPAMOCARB: MANAGING DAMPING-OFF IN PAPAYA

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B Corresponding author. Email: mike.male@deedi.qld.gov.au

INTRODUCTION

Pythium aphanidermatum is an aggressive, cosmopolitan soil borne fungal pathogen with a wide host range. In north Queensland it is economically important to the papaya industry as it causes damping-off of young papaya (Carica papaya) seedlings (1). Papaya growers wishing to establish new plantings during winter must grow containerised seedlings during the warm and often wet autumn months. These conditions are most favourable for the development of damping-off of young seedlings (2). At present, cultural controls are largely ineffective and there are no chemicals registered for the control of damping-off in containerised papaya seedlings. This paper reports on the results of two in vitro experiments which evaluated the efficacy of a range of chemicals for the control of damping-off of papaya caused by P. aphanidermatum.

MATERIALS AND METHODS

Papaya seeds of the cultivar 1B were sown in 10 cm diam. pots and thinned to 8 plants per pot following germination. At 3 weeks of age, seedlings were placed in a climate controlled experimental chamber and maintained at 30°C, >90% relative humidity and 14 hours of light per day in a randomised block design. An isolate of Pythium aphanidermatum (L. Tesoriero, pers. comm.) was revived on 2% potato dextrose agar amended with streptomycin sulfate and incubated in the dark at 27°C. Axenic cultures were produced by extracting 5mm plugs from the growing margin of the colony and placing them in potato dextrose broth in the dark at 27°C for 5 days. Mycelial mats were rinsed with distilled water, weighed and macerated in distilled water and used to inoculate each pot 48 hours after being placed in the humidity chamber. Chemical treatments (Table 1) except Bacillus+silica, metalaxyl-M as Ridomil Gold 25G® and acibenzolar-s-methyl were applied as a pot drench 3-4 h after inoculation. In experiment 1, Bacillus+silica was applied as a drench 48 h prior to inoculation. In experiment 2, it was applied at 0, 12 and 28 days after sowing. Metalaxyl-M as Ridomil Gold 25G® was incorporated into the potting mix just prior to sowing. Acibenzolar-s-methyl was applied as a seed soak 24 h prior to sowing. Untreated controls were treated with distilled water only.

Table 1. Fungicides tested for efficacy against damping-off caused by Pythium aphanidermatum.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>metalaxyl-M drench</td>
<td>Ridomil Gold 480EC®</td>
</tr>
<tr>
<td>propamocarb</td>
<td>Previcur®</td>
</tr>
<tr>
<td>furalaxyl</td>
<td>Fongard 250WP®</td>
</tr>
<tr>
<td>potassium phosphonate</td>
<td>Agrifos Supa 600®</td>
</tr>
<tr>
<td>thiophanate methyl</td>
<td>Banrot®</td>
</tr>
<tr>
<td>metalaxyl-M granule</td>
<td>Ridomil Gold 25G®</td>
</tr>
<tr>
<td>Bacillus+silica</td>
<td>Parkway</td>
</tr>
<tr>
<td>-</td>
<td>Blend®+Autofert®</td>
</tr>
<tr>
<td>acibenzolar-s-methyl</td>
<td>Boost 500SC®</td>
</tr>
<tr>
<td>dimethomorph</td>
<td>Acrobat SC®</td>
</tr>
</tbody>
</table>

Plant mortality was recorded daily from the first day of symptom expression until no further plants died.

RESULTS

Results from each experiment (Table 2) indicated that propamocarb as Previcur®, metalaxyl-M as Ridomil Gold 480EC® and furalaxyl as Fongard 250WP® provided an acceptable level of control of damping-off. Potassium phosphonate provided a variable level of control and the remainder of the treatments were ineffective.

Table 2. Efficacy of fungicides on the percentage mortality of three-week-old papaya seedlings artificially infested with Pythium aphanidermatum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>% mortality</td>
<td>(%) mortality</td>
<td></td>
</tr>
<tr>
<td>Uninoculated control</td>
<td>0.0 a</td>
<td>0.0 a</td>
</tr>
<tr>
<td>Inoculated control</td>
<td>74.6 bc</td>
<td>83.3 c</td>
</tr>
<tr>
<td>metalaxyl-M drench</td>
<td>0.0 a</td>
<td>2.08 a</td>
</tr>
<tr>
<td>propamocarb</td>
<td>0.0 a</td>
<td>2.08 a</td>
</tr>
<tr>
<td>furalaxyl</td>
<td>-</td>
<td>6.25 ab</td>
</tr>
<tr>
<td>potassium phosphonate</td>
<td>0.0 a</td>
<td>27.1 b</td>
</tr>
<tr>
<td>thiophanate methyl</td>
<td>51.1 b</td>
<td>-</td>
</tr>
<tr>
<td>metalaxyl-M granule</td>
<td>-</td>
<td>79.2 c</td>
</tr>
<tr>
<td>Bacillus+silica</td>
<td>65.4 bc</td>
<td>79.2 c</td>
</tr>
<tr>
<td>acibenzolar-s-methyl</td>
<td>80.0 c</td>
<td>-</td>
</tr>
<tr>
<td>dimethomorph</td>
<td>-</td>
<td>91.7 c</td>
</tr>
<tr>
<td>lsd</td>
<td>28.51</td>
<td>23.52</td>
</tr>
</tbody>
</table>

Means are the percentage mortality at the conclusion of the trial.
Abbreviations from same column with the same letter are not significantly different (P<0.05).

DISCUSSION

The systemic fungicides furalaxyl and metalaxyl-M have been shown to induce resistance in Pythium aphanidermatum (3) and Phytophthora palmivora (4). Metalaxyl-M is currently used in papaya at transplanting for the control of Phytophthora root rot. Due to the potential risk of biodegradation and fungicide resistance with the additional use of these chemicals, propamocarb was recommended to the APVMA for registration as a chemical control for damping-off in papaya seedlings.

REFERENCES

INTRODUCTION

Bacteria that are associated with plant roots and exert beneficial effects on plant development are referred to as plant growth-promoting rhizobacteria. They competitively colonize plant roots and can simultaneously act as a biofertilizer by enhancing plant growth and as a biopesticide by competing for nutrients or by producing antimicrobial compounds (1). This work reports on a unique, genetically distinct soil-borne strain of Bacillus subtilis, QST 713 that has been found to produce anti-fungal and anti-bacterial metabolites. This strain has previously been employed for the control of foliar diseases. More recently, research has exhibited the advantages of soil applications of QST 713 in terms of disease suppression and beneficial plant effects. The practical implications of these new findings are discussed in regards to tomatoes, potatoes and cucurbits.

MATERIALS AND METHODS

All trials run as randomized complete blocks. Squash seeds were planted in 10-cm plastic pots containing soilless potting mix, 10 replications per treatment and one plant per replication. Treatments were applied at transplanting. Squash plants were inoculated with Phytophthora capsici 3 weeks after planting. Disease severity was rated according to a rating scale of 0-5 (Table 1). Tomato seedlings were transplanted into fumigated raised beds, 30-inch (~76 cm) wide, centered 6 feet (~1.8 m) apart, 4 replications per treatment and 30 plants per replication. Six weekly drip applications were made beginning one day after transplanting. After transplants were established, tomato plants were inoculated with Rhizoctonia sp. Plant height was evaluated 5 weeks after transplant.

Two trials were run on a commercial potato farm in the Valle del Fuerte, Sinaloa, Mexico (Table 3 and Figs. 1 and 2). Treatments were applied using a motor pump backpack sprayer, at 400 litres of water/ha in furrow at planting, with 4 replications, 4 rows, 36.8 m². Evaluations were performed at harvest. Three meters/row were taken as samples and tubers were evaluated for disease incidence and weighed.

RESULTS

Table 1. Disease severity of Phytophthora on squash plants after a single treatment at planting.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>QST 713, 1.9 x 10⁷ cfu/ha</td>
<td>0.5 b</td>
</tr>
<tr>
<td>Ridomil</td>
<td>1.4 ab</td>
</tr>
<tr>
<td>Untreated</td>
<td>2.4 a</td>
</tr>
</tbody>
</table>

Table 2. Plant health effects from 6 weekly applications to soil of tomato plants inoculated with Rhizoctonia sp.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant height, cm</th>
<th>Yield kg/plot</th>
</tr>
</thead>
<tbody>
<tr>
<td>QST 713, 9.4 x 10⁷ cfu/ha</td>
<td>39 a</td>
<td>124.2 a</td>
</tr>
<tr>
<td>Ridomil</td>
<td>35.3 a</td>
<td>108.1 a</td>
</tr>
<tr>
<td>Untreated</td>
<td>34.1 a</td>
<td>92.9 b</td>
</tr>
</tbody>
</table>

Table 3. Percent incidence of soil pathogens on tubers at harvest upon treatment with B. subtilis strain QST 713 (2.9 x 10¹⁵ cfu/ha) or a Grower Standard: mixture of Trichoderma, manzoozeb, gentamycin and oxytetracycline.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>QST 713</th>
<th>Grower Std</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizoctonia solani*</td>
<td>9 a</td>
<td>23 b</td>
</tr>
<tr>
<td>R. solani</td>
<td>0 a</td>
<td>7 b</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>5 a</td>
<td>12 b</td>
</tr>
<tr>
<td>Streptomyces scabies</td>
<td>4 a</td>
<td>10 a</td>
</tr>
</tbody>
</table>

* incidence of R. solani on stems and stolons

Figure 1. Percent incidence of Fusarium sp. on tubers at harvest after in furrow treatment with B. subtilis strain QST 713 (two rates) or a Grower Standard consisting of a mixture of thiabendazole (1kg/ha) + tolclofos (5 kg/ha) and untreated control (UTC).

Figure 2. Yield of potato tubers after in furrow treatment with B. subtilis strain QST 713 (two rates) or a Grower Standard consisting of a mixture of thiabendazole (1kg/ha) + tolclofos (5 kg/ha) and UTC.

DISCUSSION

In greenhouse and open field trials, B. subtilis strain QST 713 was found to provide disease control, plant health benefits and yield improvements in both inoculated and naturally infected soils with a variety of pathogens. These results were obtained from single application at seeding as well as season long applications through drip irrigation. This represents a potential biologically based approach to address the effects of soil diseases on crops.

REFERENCES

THE POTENTIAL OF SESAME OIL EXTRACTS FOR MELOIDOGYNE JAVANICA CONTROL

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INTRODUCTION

Plant parasitic nematodes have a significant impact on Australian agricultural and horticultural crop production. Annual farm gate losses are reported to be in the vicinity of $300–450 million (1). However, available commercial nematicides pose significant risks to both end users and non-target organisms. This study examines the potential of Neotrol, a 250 g/L formulation of sesame oil as the active constituent, for nematode control.

MATERIALS AND METHODS

Eggs of Meloidogyne javanica were extracted from tomato plants by immersing roots in NaOCL (1 % available chlorine). Second stage juveniles (J2) were hatched at 26°C over a 48 hour period.

Dose response assays

Approximately 100 J2 were placed in 10 mL vials containing a range of Neotrol and water concentrations (0, 5000, 15000, 45000, 90000, 135000, 165000 and 212500 ppm of active ingredient). Nematodes were incubated at 26°C and mortality assessed at 24 and 48 h later. Nematodes were recorded as dead if they were immobile, and straight in orientation. Each treatment was replicated six times and data subject to probit analysis using PoloPlus (2).

Orientation studies

Neotrol at 250, 625, 1250, 5000 and 12500 ppm of active ingredient in WA (1%) was prepared. A glass microscope slide was used to divide a 9cm diameter Petri dish in two and one of the agar-Neotrol solutions was poured on one side. Once this had set, the glass slide was removed and water agar alone was poured to an identical depth on the other side of the plate to provide a uniform surface. Six plates were poured for each Neotrol concentration with the exception of the 12500 ppm treatment which was replicated four times. An additional six plates containing 1% WA only on each side of the slide were also prepared. Seven separate 0.2 mL drops (each containing ≈100 J2) were alternately pipetted on either side of the line dividing the treatments within a Peri dish. Plates were incubated at 26°C and movement, position and mortality of J2 were recorded after 24 h.

RESULTS AND DISCUSSION

There was a significant dose-mortality response 24 h after treatment and the estimated LC50 value did not change significantly following an additional 24 h of incubation (Table 1), indicating that most mortality occurred within 24 h of exposure.

Table 1. Dose response of M. javanica J2 to Neotrol

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>LC50 (95% CI) (ppm)</th>
<th>Slope</th>
<th>H2 (df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>1.22x10^7 (9.18x10^6-1.56x10^7)</td>
<td>4.16 (0±0.38)</td>
<td>6.29(39)</td>
</tr>
<tr>
<td>48</td>
<td>1.39 x10^3 (1.13 x10^3-1.69 x10^3)</td>
<td>5.34 (±0.64)</td>
<td>4.12(39)</td>
</tr>
</tbody>
</table>

Although Neotrol causes significant mortality of J2 within 24 h of application, the rate of Neotrol required to induce mortality is likely to be unrealistically high to be viable in the field.

In aqueous solutions, very high rates of Neotrol were required to induce mortality in M. javanica J2. However, in assays on agar plates, Neotrol concentrations as low as 625 ppm significantly affected the distribution of the nematodes, resulting in greater numbers of nematodes moving to areas of the plates which contained no Neotrol. It is possible that Neotrol is repellent to J2. Di Sanzo (3) has demonstrated that this is a mode of action of common commercial nematicides and Castro et al. (4) showed that inorganic salts can also repel nematodes. Further studies are required to more fully understand these results and to investigate the effects of Neotrol on M. javanica in the field.

REFERENCES

ADAPTED SPRING AND WINTER WHEATS WITH RESISTANCE AGAINST MULTIPLE SOILBORNE PATHOGENS (CEREAL NEMATODES – Heterodera filipjevi and Pratylenchus spp. AND CROWN ROT - Fusarium culmorum) TARGETED FOR RAINFED WHEAT PRODUCTION SYSTEMS

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INTRODUCTION

Soil Borne Pathogens (SBPs), including the Dryland Root Rot and Cereal Nematodes, cause economic yield loss in many parts of the world where cereals are the predominant cropping system and the crop is grown under sub-optimal growing conditions, particularly drought/moisture stress. In these systems, the option to use crop rotation with non-hosts is limited and, therefore, one of the most cost effective, safe and logical options is the use of genetic host resistance, whereby the inoculum of these SBPs can be reduced below economically damaging thresholds. Another challenge is that one or more of these SBPs are commonly found together and, hence, the need to multiple SBP resistance is also important. For more than 6 years, CIMMYT under the ICWIP (ICARDA CIMMYT Wheat Improvement Program) in collaboration with the Turkish Ministry of Agriculture has been actively working on the identification of resistant wheat germplasm from a range of National and International advanced wheat lines.

Work in Turkey has focussed on the Crown Rot (CR) species F. culmorum, whereas, in many other regions this and a closely related species F. pseudogarminisum have been reported and researched. CR causes significant economic losses, particularly in rainfed wheat conditions and many regions including West Asia, North Africa, Latin and North America, Europe and Australia (1). Similarly, several species of the two important Cereal Nematodes, Cereal Cyst Nematode (CCN- Heterodera spp.) and Root Lesion Nematode (RLN- Pratylenchus spp.), have been reported to have a global distribution and be economically important, particularly under drought conditions (3). Furthermore, recent studies have confirmed more widespread distribution of and, yield loss caused by both Cereal Nematodes in important wheat growing regions (4). The objective of this study was to validate and identify adapted high yielding wheats with resistance against multiple SBPs and also confirm their potential usability in other wheat growing regions.

MATERIALS AND METHODS

Since 2004, more than 2000 advanced and released spring wheat (SW) and winter wheat (WW) germplasm from Turkish National and ICWIP International nurseries have been screened against several of the SBPs and compared with a number of superior resistant lines. All SBPs (CCN – Turkish National and ICWIP International nurseries have wheat (SW) and winter wheat (WW) germplasm from several regions around the world. Since 2004, more than 2000 advanced and released spring wheat lines have been screened against SBPs and compared with a number of superior resistant lines. All SBPs (CCN – Turkish National and ICWIP International nurseries have wheat (SW) and winter wheat (WW) germplasm from various regions including West Asia, North Africa, Latin and North America, Europe and Australia. Similarly, several species of the two important Cereal Nematodes, Cereal Cyst Nematode (CCN- Heterodera spp.) and Root Lesion Nematode (RLN- Pratylenchus spp.), have been reported to have a global distribution and be economically important, particularly under drought conditions. Furthermore, recent studies have confirmed more widespread distribution of and, yield loss caused by both Cereal Nematodes in important wheat growing regions. The objective of this study was to validate and identify adapted high yielding wheats with resistance against multiple SBPs and also confirm their potential usability in other wheat growing regions.

RESULTS AND DISCUSSION

Fifteen adapted wheat germplasm have confirmed resistance to two or more SBPs (Table 1) with almost equal distribution between SW and WW. Two additional SWs are Iranian landraces which have similarly confirmed resistance to RLN for both Australia and America. As presented in Table 1, 38% of the SW are synthetic derivatives from CIMMYT International, with several offering sources of CCN resistance in regions other than Turkey and to other traits (3). Half of the winter wheats are advanced lines from the International winter wheat program of Turkey, CIMMYT and ICARDA (TCI), whilst the other four are already realised commercial cultivars in Turkey.

Table 1. Sources of wheat bread resistance against one or more SBPs (CCN, RLN and CR) identified under controlled in-vitro and field conditions in Turkey.

<table>
<thead>
<tr>
<th>SBP</th>
<th>CCN</th>
<th>RLN</th>
<th>CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCN</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>RLN</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>CR</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Significant progress has been made to both validate and identify new sources of multiple SBP resistance in adapted germplasm. Further work is underway to understand the genetic control of the resistance in some of these sources to assist pyramiding genes of resistance and to identify molecular markers. These promising lines have been used in bread wheat improvement in both National and International programs and have been distributed to more than ten countries including Australia.

REFERENCES

EFFICACY OF LOQUAT SEED TO CONTROL ROOT-KNOT NEMATODES IN VEGETABLES


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INTRODUCTION

Plant-parasitic nematodes remain a serious constraint to agricultural production worldwide. Root-knot nematode (Meloidogyne spp., RKN) is considered one of the most damaging genera of nematodes on horticultural crops and has been traditionally controlled through the use of chemical nematicides, which are toxic and may have restricted use patterns. Many producers are seeking alternative, biologically based control methods. The seeds from loquat (Eriobotrya japonica) are reported to contain cyanogenic glycosides (1) which may be able to reduce the number of plant-parasitic nematodes, and thereby reduce the damage to crops, when incorporated into the soil.

MATERIALS AND METHODS

Loquat seed was oven dried and ground to a maximum particle size of 2 mm using a grain mill. An initial glasshouse trial established efficacy against RKN in tomatoes and helped to determine rates of application for field trials. However, it also demonstrated a phytotoxicity problem if planting was carried out immediately after incorporation of the loquat seed.

Field Trial 1, 2008 A trial was established at Bundaberg Research Station on a friable, well drained Ferrosol soil which had been artificially infested with RKN (M. javanica). Treatments comprised three rates of ground loquat seed (125, 50 or 25 g m⁻², equivalent to 1250, 500 or 250 kg ha⁻¹), a chemical nematicide (Vydate L, Du Pont Australia, 240g oxamyl/L, 12 L ha⁻¹) and an untreated control. All treatments were incorporated into the soil using a rotary cultivator prior to installing trickle irrigation and covering with plastic film. The soil was irrigated within 24 hours of applying the treatments.

Tomatoes planted as seedlings 13 days after application of treatments were grown in 5 m plots as a single row supported on a trellis with an approximate spacing of 30 cm between plants, and 1.5 m between rows. Each treatment was replicated 6 times. Assessments on plant growth and nematode infestation were made at 4 weeks and 8 weeks.

Field Trial 2, 2009 A second trial was established at the same location; however, cucumbers were grown due to the prevalence of tomato leaf curl virus in the district, which had adversely affected the previous trial. Treatments were applied in the same manner as trial 1, but the rates of ground loquat seed were altered to 100, 50 and 37.5 g m⁻². Cucumbers were planted as seeds 14 days after the application of treatments, with the same spacing and replication as the previous trial. Assessments on plant growth and nematode infection were made 4 and 9 weeks after planting.

RESULTS

Field Trial 1 Four weeks after planting, the two highest application rates of loquat seed had significantly less nematode eggs per gram of root relative to the untreated plants. Similarly, there were significantly less juvenile nematodes in the soil relative to the untreated control for all rates of loquat seed (Table 1). However, there was a significant reduction in the dry shoot weight, fresh root weight and fruit weight at the highest rate (125 g m⁻²). The leaf curl virus infection confounded the final results, making it difficult to separate growth and yield losses due to RKN from those caused by the virus. At 8 weeks there were no significant differences between treatments in RKN juvenile numbers in the soil.

Table 1. Mid-trial assessment of tomatoes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RKN eggs (200 ml soil⁻¹)</th>
<th>RKN juveniles (200 ml soil⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loquat 125 g m⁻²</td>
<td>57 (5.04)c</td>
<td>38 (4.65)c</td>
</tr>
<tr>
<td>Loquat 50 g m⁻²</td>
<td>532 (7.28)b</td>
<td>82 (5.40)b</td>
</tr>
<tr>
<td>Loquat 25 g m⁻²</td>
<td>883 (7.78)ab</td>
<td>108 (5.68)ab</td>
</tr>
<tr>
<td>Vydate</td>
<td>57 (5.04)c</td>
<td>23 (4.15)c</td>
</tr>
<tr>
<td>Untreated</td>
<td>1599 (8.38)a</td>
<td>365 (6.90)a</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different at P<0.05. Numbers in parenthesis are the transformed means on which statistical analysis was conducted.

Field Trial 2 Nine weeks after planting, all rates of loquat seed significantly reduced numbers of nematode eggs on roots and juveniles in soil relative to the untreated control (Table 2). Gallling on the roots was also significantly reduced; however, there were no significant differences in yield between any of the treatments.

Table 2. End of trial assessment of cucumbers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RKN eggs (g root⁻¹)</th>
<th>RKN juveniles (200 ml soil⁻¹)</th>
<th>Gall Rating (0-10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loquat 100 g m⁻²</td>
<td>58 (4.08)jb</td>
<td>278 (5.63)jb</td>
<td>4.7 bc</td>
</tr>
<tr>
<td>Loquat 50 g m⁻²</td>
<td>84 (4.44)jb</td>
<td>199 (5.30)jb</td>
<td>5.2 b</td>
</tr>
<tr>
<td>Loquat 37.5 g m⁻²</td>
<td>84 (4.44)jb</td>
<td>169 (5.14)jb</td>
<td>4.4 c</td>
</tr>
<tr>
<td>Vydate</td>
<td>23 (3.16)jc</td>
<td>59 (4.10)jc</td>
<td>3.6 d</td>
</tr>
<tr>
<td>Untreated</td>
<td>183 (5.21)a</td>
<td>637 (6.46)a</td>
<td>6.0 a</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different at P<0.05. Numbers in parenthesis are the transformed means on which statistical analysis was conducted. * = no knots on roots; 10 = all roots severely knotted, no root system, plant usually dead.

DISCUSSION

The highest application rate of loquat seed (125 g m⁻²) caused a significant reduction in plant growth mid way through the first field experiment, suggesting a phytotoxic effect from the loquat seed. The second field experiment demonstrated that the incorporation of loquat seed at a rate between 37.5 and 100 g m⁻² had no phytotoxic effects on the growth of cucumbers. Loquat seed was not as effective at reducing nematode damage and numbers as the chemical treatment. Nevertheless, these experiments demonstrate that the application of ground loquat seed 14 days prior to planting crops, at rates between 37.5 and 100 g m⁻², may be an efficacious and safe treatment to reduce RKN numbers and the damage they cause.

ACKNOWLEDGEMENTS

Pharming Pty Ltd funded this research and provided the loquat seed. Bundaberg Research Station field staff managed the field trials.

REFERENCES

INTRODUCTION
The stunt nematode, *Merlinius brevidens* was identified in 73% of samples in a survey of the northern grain region of Australia (1). *M. brevidens* is generally not considered a major pest of cereals, however large populations (up to 55,000/kg soil) were found in association with patchy, chlorotic and stunted wheat, oat and barley crops in the region. Retrospective analysis of data from a 4-year summer crop rotation trial revealed new information about changes in population densities of this nematode.

MATERIALS AND METHODS
A 4-year summer crop rotation trial was conducted at Formartin (27.46401’S 151.42616’E) Queensland, at a field site previously managed to produce uniform high populations of the root-lesion nematode *Pratylenchus thornei* for research purposes (but which also had low populations of *M. brevidens*). The trial was a row-column design with 3 replicates; plots were 1.75 x 8 m.

In 2000 wheat (*P. thornei*-susceptible) or canaryseed (*P. thornei*-moderately resistant) were planted to establish plots with high or low populations of *P. thornei*. In November 2001 summer crops were planted (listed in Table 1). The plots were planted with the *P. thornei*-susceptible and intolerant wheat cv. Strzelecki in May 2003. Each cropping period was separated by a clean-fallow of 12 or 15 months.

Soil samples were collected to 150 cm depth before planting in 2000 and 2001 and after harvest of the summer crops in May 2002. Nematodes were extracted from soil and root samples by the Whitehead tray method, then identified and enumerated under a compound microscope. Nematode counts, expressed as no./kg of oven-dried soil, were transformed by ln(x+500) and analysed by REML in Genstat.

RESULTS
Prior to planting summer crops in November 2001 populations of *M. brevidens* in the top soil were greatest following wheat. However deeper in the soil profile, the populations peaked following canaryseed (Fig. 1).

DISCUSSION
*M. brevidens* was found throughout the soil profile and populations increased following summer crops. Although *M. brevidens* did not reduce the yield of the wheat crop planted after a 15 month clean-fallow, a crop planted immediately following the summer crops may have been affected. Further research is warranted.

ACKNOWLEDGEMENTS
Grains and Research Development Corporation

REFERENCES

INTRODUCTION
Crown rot is an important soilborne disease of winter cereals caused predominantly by *Fusarium pseudograminearum*. It is estimated to cost the Australian wheat industry an average of $79 million per annum (1). Complete resistance has yet to be reported in any wheat genotypes and hence is an ongoing issue for Australian wheat growers. Breeding for resistance to crown rot has been difficult, partly due to variability associated with disease measurement, but also due to an incomplete understanding of the nature of the genetics of resistance.

Our previous genetics work (2) found complex models of inheritance controlling crown rot resistance. This knowledge is being used to direct a number of different approaches aimed at building disease resistance levels.

Our work has found that the half-sib breeding approach captures the highest resistance levels, followed by recurrent selection.

MATERIALS AND METHODS

**Plant Material** Seven partially resistant (2-49, CPI133814, IRN497, Lang, QT10162, Sunco, and W21MMT70) and two susceptible (Puseas and Kennedy) bread wheat genotypes were used in targeted crosses.

**Seedling Trials** The seedlings were assessed for crown rot resistance in a glasshouse test, following a modification of the Wildermuth and McNamara method (3). This method is a three week duration experiment that closely mimics field inoculation, and is highly correlated with field results.

**Field Trials** Inoculated field trials were conducted, using a randomised block design. Inoculum was placed in a band lying above the seed at sowing. All plant material was harvested and assessed for stem browning and white head production at maturity.

**Genetic Methods** To better understand the resistance genes, experimentation has been conducted using the ‘generation means analysis’ quantitative genetics design, which provides a high level of detail about each cross combination. This design requires seed of the six basic generations (two parents, F1, F2, and backcrosses of the F1 to both parents of the F1). This design enables estimates of additive and dominance components of variance and heritability for each cross (4).

**Gene Pyramiding Approaches** We have used recurrent selection, half-sib breeding and DArT-directed intercrossing as methods of pyramiding resistance.

RESULTS

**Half-sib Breeding** We have used this approach to pyramid resistance from our best combining sources, as indicated by genetics experiments, into an adapted background. We have advanced this material under selection to F6, and produced resistance significantly greater than that found in 2-49, the strongest source of crown rot resistance available.

**Recurrent Selection** Using both the field and seedling testing, we have advanced populations to the F2 generation. Individual plants selected from this approach have already been found to have elevated resistance over currently available parental sources, as well as having proportions of adapted cultivars in their parentage.

**DArT-directed Intercrossing** This approach was investigating the possibility of fixing crown rot resistance in two generations of selection, using DArT markers. The DArT approach was able to show genotypic differences within resistant phenotypes, however for the material used, the gains in resistance were not as great as those provided by the previous two methods.

DISCUSSION
Our group has investigated the genetic control of crown rot resistance in many of the available sources of resistance. This information has been used to direct our crossing strategies. We have been able to avoid populations that have non-fixable resistance due to genetic models based on dominance or epistasis.

The half-sib method of gene pyramiding has proven to provide the strongest gains in resistance, and we are currently broadening our use of this method. Recurrent selection can generate strong resistance, but only when the appropriate population is chosen, otherwise gains do not remain in the fixed line.

The use of DArT-directed intercrossing did not provide gains as great as the previous two methods. This approach may gain utility when a better understanding of gene function, as it applies to crown rot, is achieved.

We have currently provided batches of seed to all wheat breeding companies in Australia, and plan to provide additional seed each year. It is anticipated that future seed releases will provide increasing levels of resistance.

REFERENCES
INTRODUCTION
Root rot of parsnip, also referred to as parsnip canker, is characterised by large, dark brown lesions, usually on the crown and upper tap root, but also on the lower tap root. Yield losses caused by the canker vary from 25 to 100%, depending on the cropping season. In Victoria, the main producer of parsnips in Australia, *Pythium* spp., *Fusarium* spp, *Phoma* spp., *Itersonilia* spp. and *Rhizoctonia* spp. have been associated with parsnip root rot (1). Very little is known about the development of disease in relation to the stage of crop development and prevailing soil and climatic conditions.

We report on a study of the succession of pathogens associated with root rot in parsnip crops grown over the period of highest disease risk (March to October) as part of investigations into disease etiology and control (Minchinton et al, unpublished).

MATERIALS AND METHODS

**Disease surveys** Systematic, monthly disease surveys were performed on parsnip roots in untreated plots of replicated field disease management trials conducted in two parsnip crops, on a sandy loam (Clyde, Victoria) and on a medium clay soil (Devon Meadows, Victoria). Both crops were sown in late March and harvested in late October 2009. Ambient temperature and soil moisture and temperature data were collected from both trial sites during the life of the crop. Sampled parsnip roots were assessed for disease incidence, which was calculated as the percentage of roots with symptoms. Disease severity was assessed on a scale of 0 to 4. Each plant was rated for symptom severity, which was calculated as a sum of scores for a symptom type.

**Pathogen identification** Pathogens were isolated from symptomatic and non-symptomatic parsnip roots sampled from untreated plots at each trial site. Sections of parsnip roots, including fine lateral roots, were washed in sterile distilled water and plated onto water agar. Oomycete-like hyphae and fungal hyphae were transferred onto V8 agar distilled water and plated onto water agar. Oomycete-like hyphae and fungal hyphae were transferred onto V8 agar respectively. Pathogen identification was performed on parsnip roots in untreated plots of replicated field disease management trials. Disease surveys included the following processes:

**Pathogen identification** Pathogens were isolated from symptomatic and non-symptomatic parsnip roots sampled from untreated plots at each trial site. Sections of parsnip roots, including fine lateral roots, were washed in sterile distilled water and plated onto water agar. Oomycete-like hyphae and fungal hyphae were transferred onto V8 agar respectively. Pathogen identification was performed on parsnip roots in untreated plots of replicated field disease management trials. Disease surveys included the following processes:

**RESULTS AND DISCUSSION**

Symptoms developed more rapidly and were more severe on the medium clay site but incidences on both sites were similar at harvest (Fig 1). *Pythium* spp. were the most frequently isolated at both sites early in the cropping season, coinciding with the coolest and relatively wet period (Fig. 2 & 3). This suggests that they were the most active and had less competition from other pathogens at the time. Fungal genera including *Phoma*, *Fusarium*, *Rhizoctonia* and *Alternaria* became more common later in the season as soil temperatures increased. Reductions in the incidence and severity of parsnip canker following soil treatments with the oomycete specific fungicide, metalaxyl at the sandy loam site, indicate a possible relationship between *Pythium* spp. and parsnip root rot. Growers may consider an earlier harvest on sandy soils to reduce the impact of secondary infections on yield by 20%.

**ACKNOWLEDGEMENTS**

The authors thank Dr James Cunnington for the identification of pathogen collections, Victorian parsnip growers for providing trial sites and HAL and the Victorian and the Federal Governments for financial support of this research.

**REFERENCES**

PROGRESS IN COMPARING *FUSARIUM PSEUDOGRAMINEARUM* INFECTION LEVELS AND CROWN ROT SYMPTOMS IN STEM INTERNODES OF CEREALS

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INTRODUCTION
Wheat (*Triticum aestivum*), durum (*Triticum turgidum* ssp. *durum*), barley (*Hordeum vulgare*), and oats (*Avena sativa*) are susceptible to infection by the crown rot pathogen *Fusarium pseudograminearum* (1). This study describes the differences in infection levels of *F. pseudograminearum* in bread and durum wheat, barley, and oat internodes using a species specific quantitative PCR (qPCR) assay and relates the average amount of fungal DNA found in the internodes to the average visual ratings of the internodes based upon a severity scale (2).

MATERIALS AND METHODS
Seedlings of the bread wheat cultivars Puseas, Sumai 3, and 2-49, the durum wheat cultivar Jandaroi, the barley cultivar Lindwall, and the oat cultivar Cleanleaf, were inoculated using the layered inoculum method of Wildermuth and McNamara (3). Internodes 1 and 2 from all tillers of inoculated and non-inoculated plants were harvested when the main tiller of each plant was at anthesis. Internodes 1 and 2 of each cultivar were dried for 24 hours in a lyophilyzer and individual stem sections were rated for disease symptoms (per cent discoloration) according to the scale of Rossi *et al.* (2). Primers were designed to elongation factor α of *F. pseudograminearum*, elongation factor g of wheat (4), elongation factor α of barley, and the tubulin gene of oats (GenBank). The quantity of *F. pseudograminearum* DNA in infected stem internodes was detected using the fluorescent dye SYTO® 9 in a qPCR assay. Mean stem score ratings, and levels of fungal DNA for internodes were calculated and analysed using one-way ANOVAs with l.s.d.'s calculated to determine treatment effects. Means with similar letters do not differ significantly (*P* = 0.05).

RESULTS

Table 1. Number of internodes analysed per cultivar.

* High rates of seedling death.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Internode 1</th>
<th>Internode 2</th>
<th>Tiller</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jandaroi *</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Puseas</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Sumai 3</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2-49</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Lindwall</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Cleanleaf</td>
<td>9</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

Jandaroi, Puseas, and Sumai 3 had significantly higher levels of per cent discoloration than 2-49, Lindwall, and Cleanleaf for internode 1 (Figure 1). Results of the qPCR assay for internode 1, showed all cultivars were significantly different from Jandaroi, and Puseas was significantly different from Cleanleaf (Figure 2). The visual scores of per cent discoloration for Internode 2 showed all cultivars were significantly different from Jandaroi, but that there were no differences among the other cultivars (Figure 1). The qPCR assay results for internode 2 showed significant differences between Jandaroi and all other cultivars (Figure 2).

DISCUSSION
The durum cultivar Jandaroi had significantly higher levels of infection than all other bread wheat, barley, or oat cultivars. Among the bread wheat cultivars, levels of infection detected in internode 1 were not significantly different, even though the visual scores indicated they were. The barley variety Lindwall was not significantly different from the bread wheat, or oat cultivars in level of infection. A low level of infection was detected in the oat cultivar Cleanleaf, and the barley cultivar Lindwall, even when visual symptoms were not evident. This study shows that qPCR can be used to understand symptom expression and resistance to crown rot.

REFERENCES
CALCULATING APPLICATION RATES FOR COMPOSTED MULCH AND SOIL CONDITIONERS TO MAXIMISE SOIL HEALTH

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INTRODUCTION

Composts can provide a stable, but slowly available source of organic carbon, capable of improving the biological and physical condition of soil. High rates of compost applied as a mulch conserve soil moisture, and can potentially replace fumigants for the control of root diseases.

Composts vary from municipal solid waste treated with worms for one month, to windrowed feedlot manure processed at 55°C for four months, marketed as mulch, potting media, topsoil or fertiliser. Despite the diversity of raw inputs and end-markets, very little information is available on calculating appropriate application rates. At worst, the use of generic composts in agriculture has resulted in seedling emergence failure and yield reductions of over 30% and 25% respectively in cotton crops, the local elimination of earthworms in a vineyard, and severe leaf yellowing in oranges (1). In this paper, a methodology for calculating agronomically objective application rates is outlined, using two case studies as examples.

CASE STUDY 1: Mulch for Avocados

Applying eucalypt mulch at 0.4 m³ per tree (14 t/ha) improved root growth and fruit size in trials in the USA and South Africa (2). A Mt Binga grower applied a pig manure/wood chip/summer forage mulch at equivalent rates, and induced severe leaf yellowing in his trees.

Table 1. Australian Standard 4454 for compost, mulch and soil conditioner test results for Mt Binga mulch, interpreted against test benchmarks in AS 4419 & 3743.

<table>
<thead>
<tr>
<th>Test and Result</th>
<th>Interpretation using AS 4419 Organic and low density soils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium 16.9 mg/L</td>
<td>&lt; 200 mg/L, low risk of ammonium toxicity</td>
</tr>
<tr>
<td>pH 6.09 5.5 – 7.5</td>
<td>considered suitable</td>
</tr>
<tr>
<td>Soluble P 16 mg/L</td>
<td>&lt; 3 mg/L for P-sensitive species.</td>
</tr>
<tr>
<td>Electrical conductivity 5.51 dS/m</td>
<td>Avocados are salt-sensitive!</td>
</tr>
</tbody>
</table>

The mulch used by the grower had been tested using the Australian Standard for compost, mulch and soil conditioners (AS 4454). Results were interpreted using equivalent tests in AS 4419 for organic and low density soils, or AS 3743 for potting mixes (Table 1). The mulch used had a very high salt and soluble phosphorus level, detrimental to the trees.

The grower continues to produce his own compost, analysing for fertiliser equivalence using tests developed for Australian soils (3). His compost is much finer than the mulch used in the published trials, and must be applied as a soil conditioner, integrating the plant-available P and K of the compost into his fertiliser management program. Soil tests are used to monitor nitrate released from the organic slow-release pool, from previous compost applications.

CASE STUDY 2: Root Disease Control in Pineapples.

Organic amendments have been applied at rates of up to 50 t/ha to reduce root knot nematodes and to control fungal root diseases (4). A cured feedlot manure and sawdust compost was applied at 50 t/ha to a pineapple crop, with no reduction in conventional fertiliser application rates. The outcome was a 34% decrease in root mass, and a 163% increase in phytophthora root disease.

Figure 1. Soil bicarbonate-extractable phosphorus and potassium and pineapple leaf nitrate and potassium concentrations. The treatments were fumigation with metham sodium, 50 t/ha feedlot manure compost, and a control. All received conventional inorganic fertiliser.

The fertiliser replacement rate for a 99 t/ha pineapple crop is 173 kg/ha for K and 14 kg/ha for P. At 50 t/ha the compost supplied 880 and 110 kg/ha respectively. At this very high rate of K, leaf nitrate levels were depressed (Figure 1), and root tips suffered from salt burn. Mycorrhizal colonisation may also have been inhibited.

Applying the compost at the reduced rate of 10 t/ha as a total replacement for fertiliser K and P, would have avoided these problems. Also the soil health outcomes associated with organic amendments should accrue, with repeat compost applications at the corrected rate, over the next 5 years.

ACKNOWLEDGEMENTS

Thanks to John Wiltshire and Graham Stirling for the use of their data.

REFERENCES

IDENTIFYING QTL FOR FUSARIUM CROWN ROT RESISTANCE
(F. PSEUDOGRAMINEARUM) IN TWO SPRING WHEAT POPULATIONS
(SUNCO / MACON AND SUNCO / OTIS)

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INTRODUCTION
Fusarium crown rot (FCR), caused by a complex of Fusarium species of which F. pseudograminearum and F. culmorum are the most important, reduces wheat yields in the Pacific Northwest (PNW) of the U.S. by an average of 9%. The advent of DNA-based markers has facilitated the application of marker assisted selection. Several significant QTL for crown rot resistance have been documented on chromosomes 1A, 1D, 2B, 3B, 4B, and 7A from resistant cultivars in Australia (1, 2, 3, 5). Bovill et al. (1) reported QTL inherited from Sunco on chromosomes 2B, 3B, and 6B in at least one glasshouse test. Our objective was to identify major quantitative trait loci for FCR resistance in Sunco through the use of two recombinant inbred line (RIL) mapping populations developed from two PNW varieties, Macon and Otis.

MATERIALS AND METHODS
Two mapping populations consisting of 151 F5:F6 and 219 F6:F7 RIL, were derived from crosses between Sunco (partially resistant) by Otis (susceptible) and Sunco by Macon (susceptible), respectively. A single PNW F. pseudograminearum isolate (006-13) collected by Smiley and Patterson (4) was used to inoculate trials conducted in several growthroom, outdoor terrace, and field assays during 2008 and 2009. Stem base crown tissues of seedlings (for the growthroom assays) or adult plants (the terrace bed and field assays) were rated for disease severity on a numeric scale from 0-10.

RESULTS AND DISCUSSION
A total of four significant QTL were identified on chromosomes 1D, 3B, 4B, and 7A with LOD scores ranging from 2.0 to 14.3. The most significant QTL was inherited from Sunco on chromosomes 3B across all three seedling growthroom assays for the Sunco/Macon population and one of the two growthroom assays for the Sunco/Otis mapping population. The maximum LOD scores of 14.3 and 10.0 explained 28% and 23% of the variation, respectively, for each of these populations. This QTL covered a 3.8 cM region and was verified in the same chromosomal location of 3B with field data in 2009 with the Sunco/Macon RIL population.

Table 1. QTL identified with each population.

<table>
<thead>
<tr>
<th>Origin/Pop.</th>
<th>Chrom.</th>
<th>Screen</th>
<th>LOD</th>
<th>LRS</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunco/SM 1D</td>
<td>Terrace</td>
<td>2.5</td>
<td>11.5</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Sunco/SM 3B</td>
<td>Growthroom</td>
<td>14.3</td>
<td>65.9</td>
<td>28%</td>
<td></td>
</tr>
<tr>
<td>Sunco/SM 3B</td>
<td>Growthroom</td>
<td>10.0</td>
<td>46.1</td>
<td>23%</td>
<td></td>
</tr>
<tr>
<td>Sunco/SM 3B</td>
<td>Field</td>
<td>2.2</td>
<td>10.1</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Sunco/SM 4B</td>
<td>Growthroom</td>
<td>2.0</td>
<td>9.2</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Otis/Otis 7A</td>
<td>Terrace 2009</td>
<td>5.1</td>
<td>23.5</td>
<td>20%</td>
<td></td>
</tr>
</tbody>
</table>

SM = Sunco/Macon; SO = Sunco/Otis

REFERENCES
INFLUENCE of soil organic matter ON soil health, soil carbon and DISEASE SUPPRESSION IN VEGETABLE CROPS

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INTRODUCTION
In order to identify the importance of organic matter on soil health and disease suppression in the short (1 application) and longer term (3 applications over 3 years), field trials with broccoli crops were set up at a large commercial vegetable property in southern Australia. These trials evaluated the impact of different organic amendments compared to the grower’s standard fertilizer and pesticide programs. In particular the trials evaluated whether addition of regular inputs of organic matter (>5-10t C/ha per crop) could build organic carbon and lead to increased benefits to soil health (ie. physical structure, chemical balance, improved biodiversity), and suppression to disease.

MATERIALS AND METHODS
The site was a sandy loam at Boneo, Victoria, with a history of clubroot disease on brassicas, caused by *Plasmodiophora brassicae*. Treatments applied in the short term study included: CaO and CaNO₃ fertilizer (standard grower practice), metham fumigation, a biofumigant (Fumifert), Shirlan fungicide, a slow release ammonium fertilizer and organic soil amendments (composted chicken manure and composted green waste). The long term study evaluated four organic treatments (composted chicken manure, composted green waste, silage and lignite) and compared these to metham fumigation and the standard grower practice. Treated soil from the field was also assessed in pots for suppression against Rhizoctonia damping off (1). Overhead irrigation, base fertiliser, insecticide and herbicides were applied as required, according to local grower practice. Trial designs were randomised complete blocks with treatments replicated 4 times. Yield data were analysed using ANOVA. Effects on soil biological, chemical and physical characteristics were also measured.

RESULTS AND DISCUSSION
The study showed that in comparison to standard grower practice without organic amendments, biofumigant products and other organic amendments generally increased yields of broccoli in both the short and long term trials (Figs 1 and 2). The organic products however differed markedly in their ability to decrease disease (Table 1). In the field, three organic amendments (chicken, green waste and lignite) promoted disease and one (silage) decreased clubroot. In pot studies, lignite, green waste and a humate tended to decrease damping off caused by Rhizoctonia (1). These differences in control were considered to be related to the C:N ratio, the rate of breakdown of the composts and the effect of the amendment on soil pH. The lack of control of clubroot was directly correlated with the decrease in pH caused by the organic treatments (Table 1).

In spite of higher disease, chicken manure gave higher and more profitable yield indicating that there were additional soil health and plant productivity benefits beyond those of the other products and possible disease suppression. Composted chicken manure, however, was shown to emit a high level of N₂O gas compared to synthetic fertilizer and other organic treatments. Present trials are aimed at finding methods which mitigate this effect to ensure that any future use of organic treatments minimises the effect on environmental and ecological balance.

ACKNOWLEDGEMENTS
The support of Horticulture Australia Ltd, DPI Victoria and the Vegetable Industry via AusVeg to this research is gratefully acknowledged.

REFERENCE

![Figure 1. Short term study: Relative average marketable yield of broccoli over 3 seasons (2008-2010) grown with different organic, fumigant and fertiliser treatments in a sandy loam at Boneo, Vic.](image-url)

![Figure 2. Long term study: Average marketable yield of broccoli over 3 seasons (2008-2010) grown with repeated yearly organic, fumigant and fertiliser treatments in a sandy loam at Boneo, Vic.](image-url)

Table 1. Long term study: Clubroot rating of broccoli grown after 3 applications of organic matter to soils (10, 5 and 5 t C/ha) over 3 seasons 2008-2010.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Clubroot rating (0-3)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>0.33</td>
<td>Low</td>
</tr>
<tr>
<td>Metham</td>
<td>0.03</td>
<td>V. low</td>
</tr>
<tr>
<td>Compost</td>
<td>0.85</td>
<td>Medium</td>
</tr>
<tr>
<td>Chicken</td>
<td>1.65</td>
<td>High</td>
</tr>
<tr>
<td>Silage</td>
<td>0.15</td>
<td>V. Low</td>
</tr>
<tr>
<td>Lignite</td>
<td>1.93</td>
<td>V. High</td>
</tr>
</tbody>
</table>
CONSECUTIVE APPLICATIONS OF BRASSICA GREEN MANURES SUPPRESS MELOIDOGYNE JAVANICA AND INCREASE YIELD OF SEMILLON GRAPE

INTRODUCTION

Root damage caused by Meloidogyne javanica in grapevines impairs nutrient and water uptake from soil, reduces vine vigour and yield. Nemacur® (as 400 g/L fenamiphos) can suppress this nematode but results from a vineyard trial also indicated significant suppression of M. javanica when Indian mustard green manure and seed meal were amended with soil (2). The suppression is thought to be due to isothiocyanates, compounds toxic to nematodes produced upon decomposition of brassica materials in soil (3). As isothiocyanates are very short lived (possibly 4-12 days) in soil (1), resurgence of pest nematodes is possible in long-term crops such as grapevines. Repeated application of brassica materials over a few consecutive growing seasons may be required to suppress M. javanica populations below damage threshold levels. Therefore, the present study was conducted to compare the effectiveness of two brassica green manures (Indian mustard cv. Nemfix and BQ™ mulch), Indian mustard seed meal and Nemacur® in suppressing M. javanica when applied in 1-3 year sequences.

MATERIALS AND METHODS

One year old Semillon rootlings were established in pots filled with sterilised soil, inoculated with 500 juveniles (J2) of M. javanica /vine and then allocated 1 of 4 treatments namely i) Mustard green manure (MGM), ii) Mustard seed meal (MSM), iii) BQ™ mulch and iv) Nemacur® with 9 vines of each. Each treatment was divided into 3 reps of 3 vines which received the treatment in a 1, 2 or 3 year application sequence. Two control treatments, diseased (no green manure) and healthy (MGM for 3-consecutive years), were also included.

Seeds of Indian mustard cv. Nemfix and BQ™ mulch at 20 kg/ha were sown in pots with the vines in early May and the brassica plants were slashed (13 t dry matter/ha) at approximately 25% flowering in September in each year. Indian mustard seed meal at 2 t/ha and Nemacur® at 30 L/ha were applied on the day when the brassica plants were slashed.

Data on nematode populations in soil and grape yield 3 years after initiation of the experiment are presented here.

RESULTS AND DISCUSSION

The population densities of M. javanica in soil and biofumigation and Nemacur® treatments were significantly lower than the population density in the diseased control treatment (Fig. 1A). This is equivalent to 37-78% nematode suppression in biofumigation and Nemacur® treated soil. A greater suppression ranging from 66-78% was observed when the biofumigation materials and Nemacur® were applied for 2-3 consecutive years (Fig. 1A). This is consistent with results from other brassica green manure and mustard seed meal studies showing ca. 90-100% suppression of M. javanica (2, 3).

The suppression of M. javanica in soil was reflected by increasing grape yield significantly when mustard and BQ™ mulch green manures were applied for 2-3 consecutive years, and mustard seed meal and Nemacur® for 3-consecutive years compared to diseased control treatment (Fig. 1B).

REFERENCES

THE ANTAGONISTIC EFFECT OF TRICHODERMA SPECIES FROM IRANIAN SOIL ON SCLEROTINIA SCLEROTIORUM, THE CAUSAL AGENT OF WHITE STEM ROT DISEASE IN OILSEED RAPE

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INTRODUCTION
About 55,000 ha of oilseed rape (Brassica napus L.) is cultivated in Golestan province in the north east of Iran. White stem rot of canola is one of the most important diseases in the north of Iran (1, 5). It is also a basic problem of rapeseed in most areas of the world (2, 4). The pathogen survives as sclerotia and then produces apothecia in soil and plant debris (4, 5). To date, biological control of this disease is an effective but controversial method of control (2, 3). In this study, native species of Trichoderma from Iran were tested against the pathogen.

MATERIALS AND METHODS
PDA medium was used to isolate Sclerotinia sclerotiorum from rapeseed fields in Gorgan. Trichoderma species were isolated from both canola and soybean fields and purified on peptone agar media (2). Four isolates of Trichoderma harzianum (99, 110, 112 and r120), one isolate of T. virens and one of T. atroviridae were identified (2, 4).

In greenhouse tests, sclerotia of S. sclerotiorum were produced on autoclaved barley seeds and added to autoclaved soil in pots (10% v/v). T. virens was produced on autoclaved wheat bran and added (5% v/v) to soil in pots (4). Rapeseed was then planted. Fresh shoot and root weights of plants were measured at various times after planting.

RESULTS AND DISCUSSION

In vitro dual culture studies showed that all Trichoderma spp. were able to inhibit growth of the pathogen. However, there were significant differences (P=0.05) in the effectiveness of T. virens and T. harzianum (m120), with the latter fungus reducing the growth of mycelia of S. sclerotiorum by 70% on PDA medium. Colonization studies showed that isolates of T. virens covered mycelia of S. sclerotiorum better than other Trichoderma spp. Volatile metabolites from T. virens inhibited growth of the pathogen by 40-50%.

Microscopic studies revealed mycelia of T. virens and T. harzianum (m120) formed appressoria and then penetrated the pathogen and coiled around hyphae. In the greenhouse experiments, rapeseed seedlings wilted in pots inoculated with sclerotia of the pathogen. Plants in control pots (without inoculation) and plants treated with Trichoderma spp. remained healthy. T. virens increased the shoot and root weight of plants inoculated with S. sclerotiorum at 2 weeks (Table 1) and at 2 months (Figure 1). Among the Trichoderma spp., T. harzianum (m120) was the only species that parasitized and destroyed sclerotia of the pathogen in media culture. Parasitized sclerotia were unviable when tested.

Table 1. Growth of canola seedlings 2 weeks after treatment with Trichoderma virens (Tv) and various isolates of Sclerotinia sclerotiorum (Ss)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot wt. (g)</th>
<th>Root wt. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not inoculated</td>
<td>85.5</td>
<td>68.8</td>
</tr>
<tr>
<td>Ss5</td>
<td>20.0</td>
<td>6.3</td>
</tr>
<tr>
<td>Ss8</td>
<td>19.5</td>
<td>8.9</td>
</tr>
<tr>
<td>Ss2</td>
<td>27.8</td>
<td>7.8</td>
</tr>
<tr>
<td>Ss5 + Tv</td>
<td>50.8</td>
<td>16.3</td>
</tr>
<tr>
<td>Ss8 + Tv</td>
<td>51.8</td>
<td>13.0</td>
</tr>
<tr>
<td>Ss2 + Tv</td>
<td>59.0</td>
<td>18.5</td>
</tr>
<tr>
<td>LSD (P = 0.05)</td>
<td>14.0</td>
<td>8.1</td>
</tr>
</tbody>
</table>

REFERENCES
VALIDITY OF COMMERCIAL SOIL HEALTH TESTS FOR VINEYARD SOILS

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South Australian Research and Development Institute, GPO Box 397, Adelaide, SA 5001
belinda.rawnsley@sa.gov.au

INTRODUCTION
In recent years there has been increasing concern about vineyard soil health. It is well documented that cover crops and the use of organic amendments, such as mulch, are beneficial to vineyard productivity by reducing weed growth, conserving soil moisture, improving biological activity and soil structure – all of which ultimately contribute to better soil health and vine performance. However, there is a distinct lack of information available to growers on how to accurately measure soil health.

Commercial services offering soil health tests are relatively new and growers are often unsure what the data actually means. The aim of the project is to investigate the validity and feasibility of commercially-available soil biological tests used to assess soil health under a range of soil management practices in the Barossa Valley.

MATERIALS AND METHODS
Vineyard selection Four vineyards were selected in the Barossa Valley, South Australia to represent common soil management practices used in the district. Vineyards (cv. Shiraz) were of the same soil type and located within 1 km of each other. Vines were drip-irrigated. The vineyards soil management practices consisted of (i) conventional cultivation (ii) undervine mulch (iii) permanent sward or (iv) biodynamic soil applications.

Soil collection Soils were sampled during the 2009/10 season in spring, summer and autumn to coincide with peak root growth. Soils were collected with a 7.5 cm auger from under the drip line approximately 40 cm away from vine trunk to a depth of 20 cm. This ensured soil was collected from the root zone. Twenty cores were randomly collected across the selected vineyard block to obtain one bulked representative sample.

Soil assessment Soils were assessed by several commercial services for physical, chemical and biological properties. Measures of soil biology included total active microbial biomass (TAMB), fungal and bacterial biomass, prokaryote biomass and free-living nematode counts. Comprehensive soil tests were also conducted to assess physical and chemical properties.

Assessment of results was based on feasibility of the test, ease of use, sampling requirements, generation and variability of data and interpretation of results pertaining to soil management practices. Viticultural practices from each vineyard site were compiled (e.g. fertiliser use, herbicides, irrigation and soil management).

RESULTS
Results from two biological tests are shown: TAMB and nematode count. TAMB for soils ranged from 0.73 – 13.12 μgC/g (Figure 1). There was consistency of results between sampling times for the three vineyards. The cultivated site soil showed less TAMB than other sites at both sampling times.

Free-living nematodes were predominant at the biodynamic site in spring 2009 then declined in the summer month (Figure 2). The cover crop and mulch vineyards were similar. Population levels were consistently lower at the cultivated site which corresponds with TAMB results.

DISCUSSION
Preliminary findings so far indicate soil biological tests are consistent over time but tests may not always correlate as expected (eg. in spring, biodynamic soil had a higher free-living nematode count than others but lower TAMB). The tests are most suitable for following changes in soil biology over time at a particular site rather than between sites. Further results are currently being interpreted for chemical and physical soil properties and their relationship to viticultural practices at each site. Overall, the findings will assist our understanding of complex soil health parameters and whether biological soil tests are valid to assess the sustainability of soil management practices.

ACKNOWLEDGEMENTS
Dr Rawnsley is the recipient of the 2009 Geoff Knights Viticulture Innovation Award, proudly supported by the Barossa Viticultural Technical Group (BVTG), Barossa Grapegrowers’ Vine Selection Society, Grape Barossa and Elders Pty Ltd. The author thanks Dr Greg Walker (SARDI) for nematode assessment.
GENETIC DIVERSITY OF FUSARIUM CULMORUM, CAUSAL AGENT OF WHEAT ROOT AND CROWN ROT DISEASES AS DETECTED BY REP-PCR MARKER

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INTRODUCTION
Crown and root rot caused by species of Fusarium are among the most widespread and damaging soil-borne diseases of wheat and barley. It is of great importance to know which species are dominant causal agents and how genetically diverse they are for breeding programs. Repetitive DNA (Rep-PCR) fingerprinting methods rely on the amplification of genomic sequences between the repetitive elements conserved in prokaryotes including repetitive extragenic palindromic (REP), and enterobacterial repetitive intergenic consensus (ERIC) elements. Our objective was to test if Rep-PCR can serve as a rapid and cost-effective method for detecting genetic diversity and production of isolate-specific fungal fingerprints in Fusarium culmorum.

MATERIALS AND METHODS
Collection of field samples and isolation of Fusarium spp. Wheat crops in eight provinces of Iran (Isfahan, Tehran, Qazvin, Ardebil, Markazi, Golestan, Mazandaran and Zanjan) were surveyed for root and crown root disease at the grain filling stage in the growing seasons of 2008 and 2009. Approximately 2 mm sections from the margin between healthy and symptomatic samples were surface-sterilized in 0.5% NaOCl solution, rinsed three times in sterile distilled water, plated on Nash & Snyder medium (Peptone 15 g, KH\textsubscript{2}PO\textsubscript{4} 1 g, MgSO\textsubscript{4}, 7H\textsubscript{2}O 0.5 g, PCNB 750 mg, Agar 20 g per liter) and incubated at 25 \textdegree C until 81 days. Monoconidial isolates were cultured on 1.5 mL tubes containing slants of SNA medium and stored at 4 \textdegree C until subsequent tests.

Identification of Fusarium spp. All the fungal materials were cultured on SNA and examined after 10 days. All the putative Fusarium spp. were grouped according to colony pigmentation on PDA, growth rate after one week on PDA and morphology of conidia and conidiophores in comparison with valid morphological identification keys. Fusarium culmorum (Fco) isolates were subjected to PCR assay using species-specific primers for Fco (1).

Genetic diversity Rep-PCR (Rep- and Eric-PCR) was used to study the genetic diversity among the Fco isolates. For statistical analyses, Rep and Eric markers were evaluated together. Similarity was calculated according to Jaccard’s coefficient. Unweighted pair-group method with arithmetical averages (UPGMA) was used to construct the dendrogram.

RESULTS AND DISCUSSIONS
Foc was the dominant Fusarium species isolated from wheat samples with crown and root rot symptoms in Iran. Foc accounted for 42 isolates (32\%) of the total isolates. The species-specific PCR assay enabled confirmation of morphological identification as a 0.57 kb band appeared for all Foc tested isolates (Fig 1). Huge polymorphic banding patterns were obtained in Rep-PCRs. PCR products ranged from 100 to 3800 bp in ERIC- and 150 to 6000 bp in REP-PCR. Total of 43 DNA fragments including 17 for REP and 26 for ERIC were used to estimate similarity among the strains. The highest similarity was observed between isolates 4 and 10 which are in concordance with their geographical distribution, as they were collected from the same province (Fig. 2). This is in line with the previous results in the neighboring countries which showed homologous geographical distribution of F. culmorum isolates (2). Our results showed that Rep-PCR is very convenient for production of isolate-specific fingerprints and suitable for genetic diversity analyses in F. culmorum. The high diversity between strains could be associated with mutations in priming sites, re-arrangements of chromosomal segments or recombination process in fungal genomes (3).

Figure 1. Agarose gel photograph showing PCR-product amplified with Fusarium culmorum species-specific primer. Lane 1 from left: 100 bp DNA size marker, lanes 2-13 F. culmorum isolates.

Figure 2. Dendrogram of similarity of 42 Fusarium culmorum isolates generated by repetitive extragenic palindromic (REP) and enterobacterial repetitive intergenic consensus (ERIC) markers. The UPGMA algorithm of NTSYS-pc and Jaccard’s similarity coefficient was used for cluster analysis.

ACKNOWLEDGEMENTS
The authors thank Iranian Research Institute of Plant Protection for financial supports.

REFERENCES
INTRODUCTION
Common root rot caused by Bipolaris sorokiniana (Sacc. In Sorok.) Shoem is an important disease of wheat worldwide. The most diagnostic symptom caused by this pathogen is a dark brown or blackened sub crown internode. Rhizospheric microorganisms such as fluorescent pseudomonad bacteria have potential to be used for biological control of the disease (1, 4). The objectives of this investigation were to determine the effects of coating wheat seeds with fluorescent pseudomonads on the severity of stem and root rot disease of wheat caused by B. sorokiniana in vitro and in vivo, and to characterize their antagonistic mechanisms for controlling the pathogen and promoting plant growth.

MATERIALS AND METHODS
Wheat plants showing root rot and necrosis on the crown were collected from several fields in Tehran province in 2008-2009. The infected tissues were surface sterilized in 0.5% sodium hypochlorite for 3-5 min and washed with sterile distilled water and placed on PDA plates containing 22-25°C for one week. Based on morphological characteristics, B. sorokiniana was identified from other related species and purified (6). Inoculum was prepared by transferring four pieces of five day old fungal culture to a 1 L Erlenmeyer flask containing 500 g wheat seeds and incubating it at room temperature for three weeks.

Isolation, selection and identification of antagonistic fluorescent pseudomonad bacteria were performed according to the previous developed methods (3,4). Direct detection of 2,4-DAPG- producing fluorescent pseudomonads in the population was conducted through extraction and amplification of DNA from individual isolates using Phl2a and Phl2b markers (3). The antagonistic potential of bacterial isolates was pre-evaluated against isolates of B. sorokiniana by using dual culture in Petri dishes containing PDA. Isolates with the most inhibition ability were selected for greenhouse testing.

Greenhouse experiment
Wheat seeds (cv. Shiroudi) were surface sterilized in 0.5% sodium hypochlorite for 5 minutes, air dried in a laminar flow and then soaked in antagonistic bacterial suspensions overnight. Inoculum of two fungal isolates (K, V) was added to the soil separately, and after 24h, 8 bacterial coated seeds were planted in each 15.5 cm plastic pot. In another treatment, the soil was drenched with suspensions of the antagonistic bacteria at 10-2-105 cfu ml-1. Pots were maintained in the greenhouse at 25°C and 90% relative humidity. Control treatments were inoculated with sterile distilled water. After 75 days, severity of the disease was recorded separately for each treatment. In addition, effects of treatments on plant height, shoot dry weight, root dry weight, shoot fresh weight, and root fresh weight were recorded.

Statistical analysis
The data were subjected to analysis of variance using MSTAT-C and the means were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION
Out of the 288 fluorescent pseudomonads tested, 215 isolates (75%) showed antagonistic effects against B. sorokiniana in vitro and 50 isolates showed inhibitory effects in vivo. According to the results of biochemical, physiological and morphological tests, the strains were identified as Pseudomonas fluorescens bv. I, III, V. In seed dressing and soil drenching tests, 35 isolates reduced the severity of the disease and increased plant height, shoot and root fresh and dry weights in the presence of the pathogen. These strains also had the most promoting effect on plant height, shoot and root fresh and dry weights of seedlings in the absence of the pathogen. The seed coating method was more efficient than the soil drenching method. Also, it was found that the 35 effective isolates were phld+ and had the ability to produce antibiotic.

Application of fluorescent pseudomonads is of interest worldwide because these bacteria produce secondary metabolites such as siderophores, antibiotics, HCN, enzymes and also induce systemic resistance. It has been reported that there is a positive correlation between population size of the biocontrol strain on roots and disease suppression (2, 5). In general, competition for nutrients supplied by roots and seeds and occupation of sites favoured for colonization probably are responsible for a small or moderate degree of disease suppression by most PGPR and are of primary importance in some strains. These results indicate that, specific rhizobacterial agents can influence disease suppression and could be considered as part of a disease control strategy like integrated pest management. However, further investigation is needed, especially in field conditions.

REFERENCES
SUPPRESSION OF PHYTOPHTHORA ROOT ROT IN PINUS RADIATA

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INTRODUCTION

Phytophthora root rot, caused by Phytophthora cinnamomi Rands and P. cactorum (Lebert & Cohn) J. Schröt., can cause serious losses in seedling production in bare-root nurseries. Infection of roots and basal stem tissues by Phytophthora spp. disrupts water uptake, leading to wilting and death of affected seedlings. This study compared metalaxyl-M and phosphorous acid (phosphite) for their ability to suppress root rot in Pinus radiata seedlings in a commercial forest nursery in New Zealand. The trial area had a recent history of phytophthora root rot where symptoms typically became more evident after root pruning, suggesting a possible link between this activity and infection.

MATERIALS AND METHODS

Trial site Trials were conducted at Te Ngae Forest Nursery, Rotorua. Pinus radiata seed (PF Olsen Ltd, New Zealand) was coated with Trichoderma spp. and with Mesurol® before being sown in beds (1 m wide and spaced 0.75 m apart) containing eight rows of seed at a density of 120/m². Trial 1 was sown on 16 October 2007 and Trial 2 on 20 October 2008. A randomised split plot experimental design was used in both trials.

Treatments In Trial 1, Ridomil® Gold MZ (a.i. metalaxyl-M+ mancozeb, Syngenta Crop Protection Ltd) was applied at 15 and 50 kg/ha (0.6 and 2.0 kg metalaxyl-M/ha), while in Trial 2, Ridomil® Gold EC was applied at 4.2 litres/ha (2.0 kg metalaxyl-M/ha). Foli-R-Fos® 400 (a.i. phosphorous acid, Key Industries Ltd) was applied at 6.6 L/ha in both trials. Application details are outlined in Tables 1 & 2.

Disease assessments and soil analysis Seedlings with drooping chlorotic needles and poor root health were recorded as having phytophthora root rot (causal agent confirmed as P. cactorum (1)). In Trial 2, soil samples were taken to determine metalaxyl half-life and to assess treatment effects on pathogen populations.

RESULTS

Four to seven applications of phosphorous acid reduced root rot incidence by 96-99% in P. radiata seedlings during 2008 (Table 1). Metalaxyl-M was ineffective at seedling emergence but reduced root rot incidence by 63% when applied shortly after root pruning.

Table 1. Root rot incidence in Pinus radiata seedlings on 30 June 2008 (258 days after sowing).

<table>
<thead>
<tr>
<th>Trial 1</th>
<th>Application timing (days after sowing)</th>
<th>Root rot %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td>22.2a</td>
</tr>
<tr>
<td>Metalaxyl-M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6 kg/ha at emergence</td>
<td>16</td>
<td>21.4a</td>
</tr>
<tr>
<td>0.6 kg/ha after root pruning</td>
<td>162</td>
<td>14.9b</td>
</tr>
<tr>
<td>2.0 kg/ha after root pruning</td>
<td>162</td>
<td>8.21c</td>
</tr>
<tr>
<td>Phosphorous acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 x (monthly, Dec.-May)</td>
<td>55-204</td>
<td>0.71d</td>
</tr>
<tr>
<td>4 x (monthly, Feb.-May)</td>
<td>121-204</td>
<td>0.82d</td>
</tr>
<tr>
<td>7 x (fortnightly, Feb.-May)</td>
<td>121-204</td>
<td>0.14d</td>
</tr>
<tr>
<td>S.E.D</td>
<td></td>
<td>1.93</td>
</tr>
</tbody>
</table>

Roots were pruned 155 days after sowing. Values followed by different letter are significantly different (P<0.001).

In Trial 2, a single application of phosphorous acid, one week before root pruning, reduced root rot incidence by 99% and was more effective (P<0.001) than metalaxyl-M, which reduced disease incidence by only 40% (Table 2). Soil analysis established that the half-life of metalaxyl in treated plots was 30 days and that none of the treatments affected the local populations of pythiaceous organisms (Phytophthora and Pythium spp.).

Table 2. Root rot incidence in Pinus radiata seedlings on 20 August 2009 (304 days after sowing).

<table>
<thead>
<tr>
<th>Trial 2</th>
<th>Application timing (days after sowing)</th>
<th>Root rot %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td>18.2a</td>
</tr>
<tr>
<td>Metalaxyl-M</td>
<td>135</td>
<td>12.6b</td>
</tr>
<tr>
<td>Phosphorous acid x1</td>
<td>135</td>
<td>0.41c</td>
</tr>
<tr>
<td>Phosphorous acid x2</td>
<td>135 &amp; 163</td>
<td>0.07c</td>
</tr>
<tr>
<td>Phosphorous acid x4</td>
<td>135, 163, 192 &amp; 219</td>
<td>0.07c</td>
</tr>
<tr>
<td>S.E.D</td>
<td></td>
<td>1.107</td>
</tr>
</tbody>
</table>

Roots were pruned 142 days after sowing. Values followed by different letter are significantly different (P<0.001)

DISCUSSION

Phosphorous acid provided superior control of phytophthora root rot in P. radiata seedlings compared with metalaxyl-M. One foliar spray of phosphorous acid suppressed root rot by 99% for at least five months. Phosphorous acid did not affect pathogen populations in treated plots and it is proposed that the chemical is translocated to actively growing tissues where it acts directly against the pathogen and potentiates host defences to attempted infection (2). Metalaxyl-M was highly effective against phytophthora root rot in laboratory trials (data not shown). However, in the field, metalaxyl-M was not effective when applied at seedling emergence and provided only moderate control when applied at root pruning. The poor field efficacy of metalaxyl may be due, in part, to its short half-life (30 days) at this site.

ACKNOWLEDGEMENTS

Thanks to the New Zealand Foundation for Research, Science and Technology (programmes LINX0304, LINX0804 & CO4X0302), to the New Zealand Forest Health Research Collaborative (projects 2007-03 & 2009-04), and Sustainable Farming Fund (L09/038) for funding. Special thanks to Warwick Brown and staff at Kaingaroa Timberlands for use of the nursery block, provision of materials and crop management.

REFERENCES


6th Australasian Soilborne Diseases Symposium, 2010
INTRODUCTION
Pythium, Fusarium, Rhizoctonia and Sclerotinia spp. are soilborne plant pathogens which cause root rot and wilt diseases of vegetables and major crop losses in vegetable production in Australia. Control of these pathogens is difficult as they produce resilient survival structures which can persist in the soil for long periods. There are currently limited IPM (integrated pest management) compatible control options for these pathogens in vegetable production.

Some plant-derived compounds have antimicrobial properties which are being investigated for potential control of plant diseases. Essential oils are a major group of volatile plant extracts, many of which have strong antimicrobial effects against some soilborne pathogens in vitro (1). This research investigated the antimicrobial effects of commercially available plant extract products on important soilborne pathogens of vegetable crops in Australia as well as the effect of plant volatiles on hyphal morphology.

MATERIALS AND METHODS
A broad range of plant extracts were screened for antimicrobial activity by conducting a series of in vitro experiments. Several treatments (14 essential oils, 4 active constituents in some essential oils and 2 commercial products) were tested in dose-dependent experiments to determine antimicrobial activity against Pythium aphanidermatum, P. sulcatum, P. irregulare, Fusarium oxysporum and Rhizoctonia solani using contact bioassays (500, 1000 and 2500 ppm). Vapour phase exposure bioassays (1, 5 and 10 μL/plate) were conducted against P. irregulare, F. oxysporum, R. solani and Sclerotinia minor. The most promising treatments from the initial screening were tested at reduced rates (1, 10, 100 and 500 ppm) to optimise their efficacy and to identify the lowest concentrations that inhibit growth or has biocidal activity against F. oxysporum, R. solani, S. minor, Pythium dissotocum complex and a beneficial soil fungus Trichoderma hamatum. The efficacy of the treatments was assessed as mycelial growth suppression and/or biocidal effect. In a separate experiment, hyphae of these 4 isolates and T. hamatum were treated with 3 essential oils at concentrations known to kill mycelium. Sections (1 mm thick) of agar plugs of mycelium were taken then mounted in lactic acid and examined using a light microscope (Olympus BX 50) to compare morphology of treated and untreated hyphae.

RESULTS AND DISCUSSION
There were significant differences (P<0.001) in mycelial growth among treatments as well as a treatment dosage interaction in both vapour and contact phases for all pathogens tested. The most effective treatments were 3 essential oils (thyme, origanum and clove bud) and 4 active compounds (thymol, geranial, eugenol and carvacrol) which completely suppressed mycelial growth and were biocidal against all pathogens tested at high dosages in contact and vapour phases (data not shown). The biocidal activity of thyme, clove bud and origanum is likely to be due to their major constituent thymol, eugenol and carvacrol respectively, which also showed biocidal activity when tested as pure compounds. These compounds have been reported to have biocidal activity to soilborne pathogens (2).

Thymol and carvacrol were biocidal to all pathogens tested at 500 ppm. Origanum was biocidal to R. solani and S. minor while thyme, clove bud and eugenol were biocidal to P. dissotocum, S. minor and R. solani respectively at 500 ppm (Table 1). Thymol completely suppressed F. oxysporum, S. minor and P. dissotocum and carvacrol suppressed R. solani and P. dissotocum at 100 ppm, but all of the mycelial plugs resumed growth on fresh media without the plant extracts. Six treatments were biocidal against some pathogens yet were not biocidal to T. hamatum (Table 1).

Table 1. The effect of plant extracts on growth of soilborne pathogens and T. hamatum (soil beneficial).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate</th>
<th>% mycelial growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>4 days</td>
</tr>
<tr>
<td>Thyme</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>Clove bud</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>Thymol</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>Eugenol</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0</td>
</tr>
</tbody>
</table>

Microscopic observations showed changes in hyphal morphology of P. dissotocum, F. oxysporum, S. minor and R. solani when treated with thyme, origanum and clove bud at rates of 500 and 1000 ppm compared to untreated hyphae. Treated hyphae appeared sunken and showed some reduction in hyphal diameter when measured under a light microscope. Other changes included collapsed cellular membranes and hyphae were emptied of cellular contents. Hyphae of T. hamatum treated with clove bud were sunken and reduced in size. However, thyme and origanum showed no changes to T. hamatum.

These results show the potential of plant extracts to inhibit growth and kill pathogen mycelium by damaging the hyphal membranes. Further experiments investigating the efficacy of plant extracts for disease control in glasshouse and field trials are currently underway.

ACKNOWLEDGEMENTS
This work was funded by the Department of Primary Industries Victoria and Horticulture Australia Ltd (HAL) using the vegetable levy and matched funds from the Australian government.

REFERENCES
INTRODUCTION
Common root rot (CRR; *Cochliobolus sativus*) occurs in all wheat growing regions of Australia and costs the wheat industry $30M annually with the potential to cause losses of up to $108M (1). Currently, resistance to CRR is assessed by visually estimating browning on the sub-crown internode (SCI) of field grown plants (2). The objective of this study is to develop a faster controlled environment (CE) method to evaluate resistance to CRR that is strongly correlated to existing field resistance classifications. The results presented in this abstract are a summary of three experiments completed to date.

MATERIALS AND METHODS
Exp 1 Four replicates of 10 wheat cultivars were grown in 7 cm square 15 cm high pots containing 330 g of pasteurised and air-dried living series Udic Pellusturt soil. Four seeds per pot were planted at depths of 2, 4, 6 or 8 cm and then the pots were transferred to a bench fitted with a self-regulating bottom watering system and under-bench heating that maintained the soil at 22°C. Date of emergence of each plant was recorded. Seven weeks after planting, the soil was washed from the roots of each plant and SCI length (SCIL) and dry weight of plant tops (TDWT) were measured. Data were analysed using ANOVA in Genstat 11th edition.

Exp 2 used similar materials as Exp 1 except that the soil was oven-dried after pasteurisation. Ten seeds per pot of 11 wheat cultivars ranging from moderately resistant (MR) to susceptible (S) were planted on a 210 g soil base layer. The band treatment added a further 90 g of soil, 0.66 g of ground wheat inoculum containing the pathogen spread evenly across the soil surface and capped with 30 g of soil. The dispersed treatment added 120 g of soil mixed with 0.66 g of inoculum. All treatments gave a planting depth of 4 cm and were replicated three times. Seven wks after planting, the soil was washed from the roots of each plant and the SCI disease severity rated (2).

Exp 3 used similar methodologies as Exps 1 & 2. Nine wheat cultivars inoculated with a band of 0.33 g or 0.66 g and replicated four times were evaluated for SCI disease severity (2) at 3, 5, 7 and 16 wks after planting.

RESULTS
Exp 1 SCIL quadrupled to 23 mm when planting depth increased from 2 to 4 cm. Planting deeper than 4 cm significantly increased SCIL by 6-11%. However, each increase in planting depth significantly \((P < 0.001)\) increased emergence time by up to 48%, reduced TDWT by up to 57% and reduced establishment by up to 20%.

Exp 2 There were significant \((P < 0.001)\) variety and inoculum placement effects. Compared to banded inoculum, dispersed inoculum increased average disease severity from 43% to 76% and reduced establishment from 91% to 72%. Banded inoculum was more correlated with field disease severity data \((r = 0.90, P < 0.001)\) than dispersed inoculum \((r = 0.66, P < 0.01)\). A significant \((P < 0.001)\) interaction between variety and inoculum placement was also identified. The increase of disease severity in the dispersed inoculum treatment was variety dependent with susceptible varieties increasing 6-12%, moderately resistant varieties increasing 15-23% and intermediate varieties increasing 27-63%.

Exp 3 There were significant \((P < 0.001)\) variety and harvest time effects. Average disease severity increased significantly \((P < 0.05)\) between each harvest. Peak correlation with field disease severity data occurred at the 5wk harvest (Fig. 1) but all harvests were significantly correlated \((3 wk: r = 0.85, P < 0.001; 7 wk: r = 0.88, P < 0.001; 16 wk: r = 0.80, P < 0.01)\). A significant \((P < 0.001)\) interaction between variety and harvest time was also identified with increasing disease severity with time on intermediate varieties but relatively stable severity on MR and S varieties.

DISCUSSION
These results have demonstrated that the development of a CE method to evaluate resistance to CRR that is correlated to existing field resistance classifications is feasible. The procedure identified in this study has the potential to improve the efficiency of CRR resistance screening by reducing the test period from >6 months to 5 wks, allowing faster delivery of results and increasing the number of lines that can be screened. Additional experiments will be conducted to verify these findings.

ACKNOWLEDGEMENTS
This research was funded by the Grains and Research Development Corporation through DAQ00142.

REFERENCES
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INTRODUCTION
Phytophthora root rot (PRR) caused by the soilborne pathogen *Phytophthora cinnamomi* (*Pc*) is a major factor limiting avocado fruit production in Australia. Zoospores infect the feeder roots causing root rot and disrupting the absorption and distribution of water and nutrients, resulting in decline and tree death. An integrated management approach is recommended, e.g. ensuring adequate drainage, mulching, gypsum, promoting active root growth etc., however, the Australian industry still relies heavily on the use of potassium phosphonate to manage PRR. Management would be enhanced by more targeted and efficient application of phosphonate, and by selection and industry adoption of PRR resistant rootstocks.

Phosphonate in ‘Reed’ avocado Potassium phosphonate can be applied as a trunk injection, bark application, or as multiple foliar sprays, with analyses to ensure adequate levels of phosphonate in roots for protection to *Pc*, and minimal residues in fruit. Most of this work has been completed with ‘Hass’ avocado. A recent trial assessed phosphonate concentrations and residues following foliar sprays, bark sprays or trunk injection application at commercial rates in ‘Reed’, which is a later-maturing variety than ‘Hass’. All treatments were first applied in May 2009, with subsequent foliar sprays in June, July and August. White feeder roots were sampled and analysed by SGS Agritech for phosphonate in September and again in December, and fruit flesh sampled and analysed in September.

RESULTS AND DISCUSSION
Assessment of rootstock material There were significant differences in tree health among rootstocks at each site (Figure 1 shows results from Duranbah trial). Selections of SRS-02, SRSR-04, ungrafted “Hass” and “Dusa™” were significantly healthier over time than most other rootstocks, many of which died during the trial. New selections continue to be evaluated, and will be made available to industry as appropriate.

DISCUSSION

![Figure 1. The effect of rootstock on health of avocado trees assessed 22 months after being planted in a high Phytophthora disease pressure site at Duranbah, NSW (tree health rating scale 0 = healthy and 10 = dead)](image)

**Phosphonate in ‘Reed’ avocado** Results show that a single injection in May gave the highest levels in feeder roots in September and December (Figure 2), when *Pc* resumes activity after winter and infects roots. However, residue levels in flesh in September are $>100$ mg/kg after trunk injection, but lower after foliar or bark sprays. This is most likely because during spring fruit are still an active metabolic sink in this later maturing variety. Subsequent trials will examine effect of later injection times on root and fruit levels. Further experimentation will also attempt to determine the “critical level” of phosphonate required in roots for good protection against *Pc*.

![Figure 2. Phosphonate concentrations in roots and flesh of ‘Reed’ avocados after foliar or trunk sprays or trunk injections of phosphonate.](image)

ACKNOWLEDGEMENTS
Funding from Horticulture Australia Ltd with levy support from Avocados Australia Ltd is gratefully acknowledged. We appreciate the cooperation from the many avocado growers involved.

REFERENCES
SYMPHYLANS MAY NEGATE THE SOIL HEALTH BENEFITS OBTAINABLE FROM ORGANIC AMENDMENTS AND ROTATION CROPS

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INTRODUCTION
Symphylans [\textit{Hansiella}\ spp. (\textit{Symphyla: Scutigerellidae})] are common soil-dwelling arthropods known to damage crops including pineapple (2) and sugarcane (3) by feeding on the root tips of young plants. Recent investigations by the ginger industry have focused on developing alternatives to fumigants and organophosphate nematicides currently used to control soil-borne disease, particularly root-knot nematode (RKN; \textit{Meloidogyne}\ spp.). The objective of this research was to determine the effects of previous cropping, tillage and amendment treatments on damage caused to ginger by symphylans.

MATERIALS AND METHODS
In September 2005, various cropping, tillage and amendment treatments were established at a site near Yandina that had previously grown sugarcane. Maize, soybean or forage sorghum were continuously cropped for green manure during summer and oats or brassica in winter under two tillage regimes [conventional tillage (CT) or minimum tillage (MT)]. A permanent pangola grass pasture and a bare CT fallow were also included. Prior to establishing the crop and pasture treatments, half the plots received a poultry manure/sawdust amendment (PS) at 100 t/ha.

Four replicate 18 m plots of each treatment were rotary-hoed in September 2009, soil was collected for nutrient analysis and the site was then planted with ginger cv. ‘Canton’. Shoot emergence was recorded from the centre 10 m of each plot. In February 2010, immature (low fibre) rhizomes suitable for confectionery were harvested from a 3 m length of row. The rhizomes were washed and weighed, roots were checked for RKN galls and were then dried, with witches brooming. Water infiltration rates (mm/min) were measured using 30 cm diameter plastic rings placed into the soil to a depth of 10 cm and filled with water.

RESULTS
Within 6 weeks of planting, it was apparent that ginger was not growing well in the pasture and continuously cropped plots, with up to 34% less shoots/m of row than treatments in fallowed soil. This difference between crop and pasture treatments and the bare fallow was also reflected in rhizome yield at harvest (Table 1).

Symphylan damage was readily apparent at harvest. It was much greater in crop or pasture treatments than in bare fallow, and tended to be most severe in PS-amended soil (Table 1). RKN caused little damage to roots or rhizomes, largely because symphylans had reduced the root biomass available to the nematode.

Levels of organic carbon were lowest in the bare fallow treatment, intermediate following crops or pasture and highest in crop or pasture soil that had been amended with PS (Table 1). Observations after harvest indicated that crop and pasture soils were well aggregated, containing many 1-2 mm diameter macropores. The fallowed soil had poor structure, resulting in very low water infiltration rates compared with other treatments (Table 1).

Table 1. Impact of rotation, tillage and amendment treatments on severity of symphylan damage, yield of ginger, soil organic carbon and water infiltration. Means followed by the same letter within columns do not differ significantly ($P=0.05$).

<table>
<thead>
<tr>
<th>Rotation/tillage/amendment</th>
<th>Rating (0-5)</th>
<th>Rhizome wt (kg)</th>
<th>C$_{org}$ (%)</th>
<th>Water infiltration (mm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallow/CT/Nil</td>
<td>1.00 a</td>
<td>3.33 a</td>
<td>2.61</td>
<td>14 b</td>
</tr>
<tr>
<td>Crop/CT/Nil</td>
<td>2.75 b</td>
<td>2.99 a</td>
<td>2.80</td>
<td>134 a</td>
</tr>
<tr>
<td>Crop/CT/PS</td>
<td>2.75 b</td>
<td>2.65 a</td>
<td>3.21</td>
<td>113 a</td>
</tr>
<tr>
<td>Crop/MT/Nil</td>
<td>2.25 ab</td>
<td>2.02 ab</td>
<td>2.79</td>
<td>87 a</td>
</tr>
<tr>
<td>Crop/MT/PS</td>
<td>3.25 b</td>
<td>1.19 b</td>
<td>3.91</td>
<td>74 a</td>
</tr>
<tr>
<td>Pasture/Nil</td>
<td>2.25 ab</td>
<td>2.39 ab</td>
<td>3.33</td>
<td>79 a</td>
</tr>
<tr>
<td>Pasture/PS</td>
<td>2.75 b</td>
<td>2.44 ab</td>
<td>3.64</td>
<td>122 a</td>
</tr>
</tbody>
</table>

DISCUSSION
Based on criteria commonly used to evaluate soil health (e.g. organic carbon levels, physical structure, suppressiveness to pathogens), cropped and pasture soils were much healthier than soil that had been fallowed for 3 years. However, they did not support better ginger growth, largely because their open structure was favourable to symphylans and facilitated their damaging feeding behaviour. Bioassays in September of 2006-2008 indicated that crop and pasture soils were much more suppressive to RKN than the fallowed soil, and that suppressiveness was enhanced by minimum tillage and the PS amendment (1). Damage from symphylans was particularly severe in PS-amended soil, possibly because the amendment improved crop and pasture growth and the greater root biomass supported higher populations.

This result highlights the difficulties involved in managing a complex of soilborne diseases in ginger. Crop rotation and organic amendments will improve a soil’s physical and chemical status and should reduce losses from RKN, Fusarium wilt and Pythium rhizome rot (1), but increased symphylan populations may reduce yield and negate these benefits.

Symphylans often cause problems in organic production systems (4) and are likely to become more important pests as practices designed to improve soil health are used more widely. Future research should be directed towards understanding the role of parasites and predators of symphylla (e.g. fungi, mites, beetles, spiders and centipedes) in regulating populations of the pest.

ACKNOWLEDGEMENTS
We thank Cecil Davison and ACIAR for their support.

REFERENCES
DECOMPOSING CROP RESIDUES ENHANCE SUPPRESSIVENESS TO PLANT-PARASITIC NEMATODES IN SUGARCANE SOILS

G R Stirling, M J Bell and N V Halpin

INTRODUCTION
The process of green cane trash blanketing (where sugarcane is harvested green and crop residues are retained on the soil surface as mulch) has been widely used in the Queensland sugar industry since the mid 1980s. This paper reports on the role of the trash blanket in enhancing suppressiveness to plant-parasitic nematodes.

MATERIALS AND METHODS
Data were collected from a first ratoon crop of sugarcane growing in a sandy loam soil near Bundaberg, Queensland. Root distribution in the 0-20 cm zone was measured in March 2008 (mid season) and to a depth of 1.5 m in October 2008 (immediately after harvest). Root health was assessed using ratings of 1-5, where 1 = no healthy fine roots and 5 = a uniform mass of healthy fine roots constituting a major proportion of total root length. Nematodes were recovered from samples taken at various depths in the profile and C and N were measured in the upper layers of soil.

RESULTS
Sampling to a depth of 1.5 m indicated that 85% of root biomass was in the upper 20 cm of the profile and that the population density of plant-parasitic nematodes (PPN) was not related to root distribution (Fig. 1).

Samples taken in March 2008 indicated that a large proportion of the roots were concentrated just under the trash blanket (Table 1) and that these roots showed few signs of the lesions and blackening usually observed on sugarcane roots. Roots in the 0-2 cm zone also had fewer plant-parasitic nematodes than those further down the profile (Table 1). In contrast, C and N levels and the number of free-living nematodes were highest in the surface soil and declined with depth (Table 2).

Six months later, the distribution of roots and nematodes in the 0-20 cm zone was similar to the previous sample (data not presented). A dense layer of fine roots was still apparent under the trash blanket, with roots in the 0-2 cm zone having a fine root rating of 4.4 compared with 2.4, 1.6, 1.5 and 1.4 at depths of 2-5, 5-10, 10-15 and 15-20 cm, respectively.

DISCUSSION
Our data indicate that a large proportion of the roots of sugarcane (particularly the fine roots responsible for water and nutrient uptake) are located in the region immediately below the trash blanket. These roots are also much healthier than those further down the profile, and harbour fewer parasitic nematodes.

Root-knot and lesion nematodes are obligate parasites of plants and their distribution with depth would be expected to mirror root distribution. The low nematode population densities observed in the 0-2 cm zone therefore suggest that surface soil is much more suppressive to these nematodes than the soil below it.

Since soil C levels were much higher in surface soil than at depth and the proportion of labile C to total C declined from 12% just under the trash blanket to about 8% at 15-20 cm, we suggest that C inputs from the trash blanket are involved in sustaining suppressiveness. We hypothesise that the trash blanket ‘drip feeds’ labile C into the soil, helping to sustain an active and diverse soil food web that is responsible for suppressing root pathogens and maintaining root health.

The main message from this work is that the trash blanket plays a vital role in maintaining root health. Not only does it improve the environment for root growth by reducing fluctuations in moisture and temperature but it also provides some of the C inputs required to sustain a soil food web capable of suppressing pathogens. These beneficial effects should be even greater when rotation crops are introduced to decrease populations of pathogens and losses of soil C are reduced by minimising tillage (1).

The main impediment to progress is the propensity of some growers to see crop residues as a revenue source. Instead of retaining the trash blanket and using it as a means of improving soil health, residues are being transported off-farm for use as fuel, mulch and other products.

REFERENCES
IDENTIFYING AND DEVELOPING SOILS THAT ARE SUPPRESSIVE TO PYTHIUM RHIZOME ROT OF GINGER

G R Stirling*, J P Smith†, S D Hamill§ and M K Smith||

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INTRODUCTION
Rhizome rot of ginger caused by *Pythium myriotylum* was first encountered in Australia during the wet summer of 2007/08 (1). Since then, disease epidemics have been observed on several farms and in 2010, yield losses contributed to a reduced intake of processing ginger. This paper shows that ginger-growing soils contain microbial communities capable of suppressing rhizome rot and also demonstrates that the level of suppressiveness to the disease is affected by the way soil is managed.

MATERIALS AND METHODS
In September 2005, various cropping, tillage and amendment treatments were established in a sandy clay loam soil (Grey Dermosol) on a ginger farm near Yandina, Queensland. This site was free of ginger pathogens because it had previously grown sugarcane. A similar set of treatments was included in an experiment established in April 2006 on a light clay soil (Red Ferrosol) on a farm near Kandanga, Queensland. The continuous cropping treatment consisted of growing maize, soybean or forage sorghum during summer and oats or brassica during winter under two tillage regimes [conventional tillage (CT) or minimum tillage (MT)]. There was also a permanent pangola grass pasture, together with a bare fallow that was cultivated regularly to control weeds at Yandina or a CT pangola grass pasture, together with a bare fallow that was disturbed or diminished (i.e. fumigated and bare fallow soils) had significantly lower levels of suppressiveness compared to the Crop/MT/PS treatment. These results demonstrate that the way soil is managed influences its suppressiveness to *Pythium* rhizome rot. It should therefore be possible to reduce the impact of this disease by modifying the current ginger farming system.

RESULTS AND DISCUSSION
Leaf yellowing began within 2 weeks of inoculation and symptoms then increased in severity for the duration of the experiment. Less than 5% of the 216 non-inoculated assessments were symptomless. Disease severity was much greater in non-irradiated (NIR) soils than irradiated (IR) soils and both (Table 1), indicating that disease severity was high in both soils after they were irradiated. It is therefore possible that the soil microorganisms which were inhibiting *Pythium* (through competition, antibiosis or parasitism) were more active in acidic soils.

Soils amended with PS and cropped soils that were subject to minimal disturbance (MT) were most suppressive to rhizome rot (Table 2). Conversely, treatments where the soil microbial community was disturbed or diminished (i.e. fumigated and bare fallow soils) had significantly lower levels of suppressiveness compared to the Crop/MT/PS treatment. These results demonstrate that the way soil is managed influences its suppressiveness to *Pythium* rhizome rot. It should therefore be possible to reduce the impact of this disease by modifying the current ginger farming system.

**Table 1. Impact of irradiation and soil type on the severity of Pythium rhizome rot in ginger**

<table>
<thead>
<tr>
<th>Site</th>
<th>Irradiated</th>
<th>Non-irradiated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kandanga</td>
<td>2.71 c</td>
<td>2.28 b</td>
</tr>
<tr>
<td>Yandina</td>
<td>2.56 bc</td>
<td>1.38 a</td>
</tr>
</tbody>
</table>

Values (disease severity ratings after 3 months) followed by the same letter are not significantly different (P = 0.01)

**Table 2. Impact of rotation, tillage and amendment treatments on suppressiveness to Pythium rhizome rot**

<table>
<thead>
<tr>
<th>Rotation/tillage/amendment</th>
<th>Disease severity</th>
<th>% plants remaining symptomless</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fumigated/CT/Nil</td>
<td>2.65 b</td>
<td>0</td>
</tr>
<tr>
<td>Crop/CT/Nil</td>
<td>2.25 ab</td>
<td>6</td>
</tr>
<tr>
<td>Crop/CT/PS</td>
<td>2.05 ab</td>
<td>9</td>
</tr>
<tr>
<td>Crop/MT/Nil</td>
<td>2.35 b</td>
<td>17</td>
</tr>
<tr>
<td>Crop/MT/PS</td>
<td>1.68 a</td>
<td>24</td>
</tr>
<tr>
<td>Pasture/Nil</td>
<td>2.60 b</td>
<td>0</td>
</tr>
<tr>
<td>Pasture/PS</td>
<td>2.41 b</td>
<td>5</td>
</tr>
</tbody>
</table>

For each site, numbers in each column followed by the same letter are not significantly different (P = 0.05). Disease severity was assessed in non-irradiated soils after 3 months.

REFERENCES
INTRODUCTION

Ginger (Zingiber officinale) is a small but important crop in south-east Queensland. About 8,000 t of ginger is produced each year from 220 ha of land, generating $20 million in farm-gate value and an additional $60 million from value-adding into confectionary ginger. Land used for ginger production is farmed intensively. The soil is cultivated regularly and aggressively and a typical annual cycle (early-harvest ginger followed by a winter green manure crop of oats or brassicas) often continues for several years before a field is rested with a pasture break.

This work was a component of a project investigating more sustainable methods of growing ginger. It examined the likely impact of changes to the farming system on suppressiveness to Meloidogyne spp. and Fusarium oxysporum f. sp. zingiberi, the most important soilborne pathogens of ginger.

MATERIALS AND METHODS

Various cropping, tillage and amendment treatments were established in September 2005 in a field on a ginger farm near Yandina that was free of ginger pathogens because it had previously grown sugarcane. In the continuous crop treatments, maize, soybean or forage sorghum were grown during summer and oats or brassicas during winter under two tillage regimes [conventional tillage (CT) or minimum tillage (MT)]. A permanent pasture of pangola grass was also established, together with a bare fallow that was cultivated regularly to control weeds. Prior to establishing the crop and pasture treatments, half the plots received a poultry manure/sawdust amendment (PS) at 100 t/ha.

In the following three years (September 2006, 2007 and 2008), soil was collected from 4 replicate plots of each treatment and assessed for suppressiveness. In the root-knot nematode assay, tomato seedlings were planted in potted soil and inoculated with 6,000 eggs of M. javanica. After 7 weeks, roots were rated for galling (0-10 scale) and nematode eggs were retrieved using NaOCl. For the Fusarium assay, ginger was planted in each pot and inoculated with the pathogen to achieve 0, 10⁴ or 10⁵ inoculum densities. 

RESULTS

Results from the root-knot nematode assay in year 2 are presented in Table 1. Fallowing with conventional tillage reduced soil C levels and resulted in the highest gall ratings and final nematode population densities. Cropped and pasture soils were suppressive to root-knot nematode, with suppressiveness tending to increase under minimum tillage or in soil that had been amended with organic matter.

Similar trends were observed in years 1 and 3, with analyses of the factorial component of the experiment consistently showing significant effects of treatments on suppressiveness (pasture > MT crop > CT crop, and PS amendment > nil). In year 3, an additional experiment showed that results from the suppression assay were similar, whether eggs, egg masses or second-stage juveniles of M. javanica were used as inoculum.

<table>
<thead>
<tr>
<th>Rotation/tillage / amendment</th>
<th>Labile C (g/kg)</th>
<th>Gall rating</th>
<th>Eggs/plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallow/CT/Nil 1.3 f 5.3 a</td>
<td>298,540 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crop/CT/Nil 2.1de 5.0 a</td>
<td>202,770 ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crop/CT/PS 2.8 c 4.5 ab</td>
<td>107,650 abc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crop/MT/Nil 1.9 e 4.2 ab</td>
<td>82,220 bc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crop/MT/PS 3.2 b 3.7 b</td>
<td>36,310 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasture/Nil 2.3 d 3.7 b</td>
<td>60,260 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasture/PS 3.7 a 3.5 b</td>
<td>49,550 c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

These results add to a body of evidence (1) which indicates that biologically active soils are suppressive to root-knot and other parasitic nematodes. Although Meloidogyne eggs are more susceptible to antagonists when freed from their protective gelatinous matrix (2), our results with egg masses and juveniles suggest that the suppressiveness observed was not an artefact of the assay system. One unexpected result was the long-term effect of the poultry manure/sawdust amendment, which was still having an impact more than 3 years after it was applied.

Although rotation cropping and other soil management practices did not affect suppressiveness to F. oxysporum f.sp. zingiberi, a 3-year break from ginger is still likely to be useful in managing this pathogen, as inoculum densities will decline during the break, thus reducing disease severity. When minimum tillage and inputs of organic matter are also incorporated into the farming system, soil physical, chemical and biological properties should improve and suppressiveness to root-knot nematode will be enhanced.

ACKNOWLEDGEMENTS

We thank Cecil Davison and ACIAR for their support.

REFERENCES

STUDIES ON THE EFFECTIVENESS OF TRICHODERMA AND SOIL AMENDMENTS AGAINST STEM AND POD ROT CAUSED BY SCLEROTIUM ROLFSII IN GROUNDNUT

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INTRODUCTION

Stem and pod rot caused by Sclerotium rolfsii Sacc is an important pathogen of groundnut, inducing a variety of symptoms including seed rot, seedling blight, collar and stem rot, peg rot and pod rot. Yield losses of over 25 percent have been reported (4). The continuous use of fungicides to control stem rot can cause soil and air pollution, and is hazardous for humans, animals and beneficial rhizosphere microorganisms. Therefore, methods of chemical control other than chemical control have been given alot of importance in recent years. The present study was conducted to find non chemical alternatives to fungicides for managing stem and pod rot.

MATERIALS AND METHODS

A field experiment was conducted to determine the effect of bio-agent (Trichoderma sp.), gypsum and fly ash alone and in combinations on severity of stem and pod rot. Groundnut cultivar, JL-24, susceptible to Sclerotium rolfsii was sown in 5 x 3 sq. m infested plots during the rainy season in 2006 and 2007. The experiment was laid out in a randomized block design with three replications. The commercial product of Trichoderma sp. (4 g/kg seed) was used for seed treatment (ST). Gypsum (500 kg/ha) and fly ash (30,000 kg/ha) were applied to soil (SA) prior to sowing. Carbendazim + Mancozeb 75% WS (3g/kg seed) was also included as a standard check and proper control plots were duly maintained. For stem rot, all healthy and diseased plants were counted and for pod rot, ten diseased plants were physically examined for number of pods showing complete or partial rotting was recorded. The pod and fodder yield were also recorded.

RESULTS AND DISCUSSION

The control plot recorded stem rot and pod rot incidences of 17% and 19%, respectively. The results presented in the Table 1 revealed that all the treatments significantly reduced the incidence of stem as well as pod rot. The reduction was in the range of 18-56% and 22-60% for stem rot and pod rot, respectively. Highest reduction was noticed in Trichoderma+ Gypsum plots (56% and 60%) followed by Carbendazim + Mancozeb (55% and 59%) and Trichoderma + Fly ash (50%) and 58%) for both stem rot and pod rot, respectively. The reduction in incidence and severity of stem and pod rot brought about marked increase in the groundnut dry pod and fodder yield (Fig 1). Maximum increasing in pod yield (35%) and fodder yield (62%) was observed in Trichoderma+Gypsum followed by Trichoderma+Fly ash (31% and 54%).

Table 1. Effect of different non chemicals on percent disease control of stem and pod rot of groundnut.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent disease control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stem rot</td>
</tr>
<tr>
<td>Trichoderma (ST)</td>
<td>46.4</td>
</tr>
<tr>
<td>Gypsum (SA)</td>
<td>25.5</td>
</tr>
<tr>
<td>Fly ash (SA)</td>
<td>18.7</td>
</tr>
<tr>
<td>Trichoderma (ST)+Gypsum (SA)</td>
<td>55.7</td>
</tr>
<tr>
<td>Trichoderma (ST)+Fly ash</td>
<td>50.5</td>
</tr>
<tr>
<td>Carbendazim+Mancozeb 75% WS (ST)</td>
<td>54.5</td>
</tr>
<tr>
<td>CD&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td>2.2</td>
</tr>
</tbody>
</table>

The reduction in stem and pod rot incidence in the presence of Trichoderma sp. could be attributed mainly due to antibiosis or hyperparasitism. Some chemical substances were released by Trichoderma, such as non-volatile sesquiterpene antibiotic Trichoderma 1, 2 and 3 which could inhibit the growth of Sclerotium (2). High levels of calcium in plant tissues partially off-set the action of oxalic acid and cell wall degrading enzymes produced by the pathogen. Soil application of gypsum reduced the stem rot and increased the groundnut yield (3). The present work confirms the utility of bio-control of Sclerotium rolfsii in groundnut (1) and also by Carbendazim + Mancozeb 75% WS. However, as indicated by Upadhyay and Mukhopadhyay (5), integration of cultural, biological and chemical means of control is a promising way of controlling soil-borne pathogens. The results suggest that gypsum is useful and in fact, it is already a popular practice among farmers as Trichoderma and gypsum or fly ash can easily be integrated into a management system that prevents Sclerotium rolfsii from posing a threat to groundnut cultivation. Thus, the present work records effective management of stem and pod rot by the integration of biological and cultural methods in addition to the present use of Carbendazim + Mancozeb 75% WS for the management of disease.

REFERENCES


Figure 1. Effect of different disease treatments on percent increase in pod and fodder yield of groundnut.
INTRODUCTION

Plants exhibit a variety of responses during infection by pathogens, insects, or abiotic stresses, many of which involve the activation of host defense genes. Activation of these genes leads to physical and biochemical changes in plant cells which are not favourable for damage progress in plant. Among the major biochemical changes are biosynthesis and the accumulation of inducible defense-related proteins. Most of these proteins correspond to pathogenesis-related proteins (PRs). Root rot of sugar beet, caused by the destructive soil borne fungus *Rhizoctonia solani* intraspecific groups AG2-2 IIIB and AG2-2 IV, is one of the most important and destructive diseases of this crop causing high yield losses worldwide every year. Therefore, understanding the mechanisms involved in basal resistance of sugar beet against this pathogen is necessary to plan better strategies for disease management.

MATERIALS AND METHODS

In the present study, we investigated peroxidase activity as described by Garcia et al. (1) in a partially resistant sugar beet cultivar, Ramona, after challenge inoculation with *R. solani* (2). In this cultivar, which shows a high level of resistance to the disease, peroxidase activity was significantly increased after challenge inoculation with the pathogen. In a leaf disc assay, the effect of potassium cyanide (KCN) as a peroxidase on the level of disease resistance in Ramona cultivar was determined.

RESULTS AND DISCUSSION

A clear negative correlation was observed between disease progress and peroxidase activity in the leaf discs treated with KCN. Application of potassium cyanide (KCN) as a peroxidase inhibitor significantly increased the disease progress on Ramona plants. Peroxidases belong to the PR-9 family of pathogenesis-related proteins. They are key enzymes in the cell wall-building process, and it has been suggested that extracellular or wall-bound peroxidases would enhance resistance against various pathogens by the construction of a cell wall barrier that may hamper pathogen ingestion and spread in plant cells. Our results revealed the important role of peroxidase in basal resistance of Ramona cultivar to *R. solani*. High level of peroxidase activity in this partially resistant sugar beet cultivar is an effective defense mechanism against the root rot pathogen. Therefore, using transgenic sugar beet cultivars expressing peroxidase gene can be a novel strategy to control the root rot pathogen.

REFERENCES


Figure 1. Peroxidase activity at various time points after challenge inoculation of sugar beet with *R. solani*. 

6th Australasian Soilborne Diseases Symposium, 2010
MOLECULAR AND CYTOLOGICAL ASPECTS OF TOMATO-RHIZOCTONIA SOLANI INTERACTION

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INTRODUCTION
Plants naturally express variable levels of resistance against different groups of their pathogens. This kind of primary defense response is known as basal, general, partial, polygenic or multigenic resistance that is controlled by several genes. Basal resistance partially protects plants against challenge infection by pathogens and decreases the progress and destructive effects of disease. It is obtained by cooperation of multiple molecular and cellular defense responses and involvement of various signaling pathways.

MATERIALS AND METHODS
In this study, the molecular and cellular changes of a partially resistant (Sunny 6066) and a susceptible (Rio Grande) tomato cultivar after infection with necrotrophic soil-borne fungus *Rhizoctonia solani*, causing seedling damping-off, were compared. The expression of defense related genes such as chitinase (LOC544149) and peroxidase (CEVI-1) in infected tomato cultivars was investigated using semiquantitative reverse transcription-polymerase chain reaction (RT-PCR). Furthermore, we investigated formation of phenolic compounds, such as lignin, which plays an important role in the resistance of plants to pathogen attack because of belonging to the antimicrobial defense arsenal.

RESULTS AND DISCUSSION
RT-PCR analyses revealed considerably elevated levels of expression for both genes in the partially resistant cultivar compared to the susceptible cultivar. Cytological observations of infected tomato seedling samples revealed that lower level of disease symptoms in the partially resistant cultivar is associated with decreased plant colonization by the pathogen. One of the most prominent facets of basal plant defense responses is the formation of physical barriers at sites of attempted fungal penetration. These structures are produced around the sites of potential pathogen ingress to prevent pathogen progress in plant tissues. We investigated formation of phenolic compounds, which play an important role in the resistance of plants to pathogen attack because of belonging to the antimicrobial defense arsenal. A correlation was found between accumulation of lignin and higher level of resistance in Sunny 6066 compared to Rio Grande cultivar. These findings suggest the involvement of chitinase, peroxidase, and phenolics production in defense responses of tomato plants against *R. solani* as a destructive phytopathogen.

REFERENCES

Figure 1. Time-course study of defense related gene expression at various hours post- inoculation (hpi) in two cultivars of tomato.
INVOLVEMENT OF PHENYLPROPANOID SIGNALING IN DEFENSE RESPONSES OF SUGAR BEET TO A NECROTROPH PATHOGEN

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INTRODUCTION
Root rot and foliar blight diseases caused by Rhizoctonia solani AG2-2 IV are serious threats for sugar beet production worldwide. Investigating the defense mechanisms of sugar beet plants to this soil borne necrotrophic fungus can be helpful for planning successful strategies to control the destructive Rhizoctonia diseases. In this study, we examined the expression of phenylalanine ammonia-lyase (PAL), as the first gene in the phenylpropanoid signaling pathway, at various time points after challenge inoculation in plants treated with a defense activator (riboflavin) and control plants treated with water.

MATERIALS AND METHODS
Total RNA was isolated from frozen sugar beet leaves using TRIzol reagent following the manufacturer’s instructions and subsequently treated with RNase-free DNase to remove contaminating DNA. RNA concentration was quantified by spectrophotometry before and after DNase treatment. RNAs were reverse-transcribed using oligo dT primers and SuperScript reverse transcriptase. A gene-specific primer pair was designed with Beacon Designer 4.0 (Premier Biosoftware International) for amplification of the cDNA fragment of the PAL gene. The qRT-PCR amplifications were carried out in triplicate in 96-well plates with a total volume of 25µL, as described previously (2).

RESULTS AND DISCUSSION
An increase in the PAL expression was observed at 8 hpi and it reached to its maximum expression level at 16 hpi (Fig. 1). Induction of PAL gene was slowly decreased thereafter in riboflavin-treated inoculated sugar beet leaves. In mock-treated inoculated control plants, a slow induction of the PAL expression was observed which was followed by a moderately sharp increase at 72 hpi. At this time point post inoculation, the first leaf of six sugar beet plants was collected, pooled and subjected to RNA extraction for using in qRT-PCR.

REFERENCES
HYDROPONICS ENABLES PRECISE IDENTIFICATION OF INFECTION WINDOW IN COMMON SCAB DISEASE OF POTATO

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INTRODUCTION

Common scab caused by pathogenic Streptomyces spp. is one of the most important diseases of potato worldwide, with epidemics being sporadic and strongly influenced by environmental conditions (1). Infections are thought to occur during early tuber development (2, 3) yet in field and pot-based systems precise identification of tuber initiation and development is difficult as tubers are underground, necessitating destructive monitoring processes. The objectives of this study were to develop a methodology enabling precise identification of tuber development and successful infection of tubers in a non-destructive manner. A hydroponic system was used to generate tubers in a soil-less environment with pathogen inoculation used to correlate the efficiency of infection with tuber development.

MATERIALS AND METHODS

Plant establishment and treatments

Potato plants of the variety ‘Desiree’ grown from tissue culture were transplanted into the hydroponic setup (4) (Figure 1). Sprays of S. scabiei inoculum were made to tubers of known age, with tubers sprayed at 3 different physiological ages.

Disease assessment

At senescence, percentage of tubers with lesions were recorded, individual tubers were also scored for lesion coverage (incidence) and depth (severity) (1).

RESULTS AND DISCUSSION

S. scabiei sprays on developing hydroponically-grown tubers were able to induce common scab symptom development (Figure 2) in the susceptible variety ‘Desiree’. Tuber infection rates were higher in the spring trial where double inoculations per treatment were used (Figure 3). The highest percentage infection was 36.6% when inoculated 20 DAT in trial 1, and 66.6% when inoculated at 3 and 8 DAT in trial 2. Inoculation of more mature tubers (trial 1 – 30 DAT, trial 2 – 23 and 28 DAT) showed a reduction in symptom expression suggesting reduced susceptibility, perhaps due to increased physical resistance e.g. suberisation of lenticels (2). These results showing infection was greatest during the early stage of tuber formation are in agreement with others (2,3). However, the current study more accurately quantifies tuber age at inoculation date as tubers are visible and their initiation can be observed directly in a non-destructive manner.

Figure 1. Schematic diagram of hydroponic setup.

Figure 2. Typical common scab disease symptoms on cv. Desiree at harvest after inoculations with a spore suspension of S. scabiei at 13 and 18 days after tuber initiation.

Figure 3. Percentage of tubers infected with common scab after treatment of tubers with S. scabiei spray suspension at various tubers ages i.e. days after tuberization (DAT).

The establishment of disease in artificial soil-less media represents an alternative strategy for studying parameters like tuber physiology on disease development and thus provides complementary technology to pot and field based studies for better understanding the common scab pathosystem.

ACKNOWLEDGEMENTS

This work was facilitated by Horticulture Australia Ltd in partnership with the Potato Processing Association of Australia, and was funded by the potato levy.

REFERENCES

2,4-DICHLOROPHENOXYACETIC ACID INDUCED RESISTANCE TO COMMON SCAB OF POTATO

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INTRODUCTION

Common scab, caused by the infection of developing tubers by *Streptomyces* spp., is one of the most economically important diseases of potato in Australia, yet there are no practical control methods. Previous studies have shown that 2,4-Dichlorophenoxyacetic acid (2,4-D) and related chemicals can reduce symptoms of common scab when applied to the foliage of potato (1, 2). The mechanism by which 2,4-D induces disease resistance is unclear, but recent evidence suggests direct competition with a phytotoxin, thaxtomin A, which is produced by the pathogen and essential for disease induction (1).

However, in addition to a reduction in symptoms 2,4-D was also shown to decrease tuber size, increase tuber number, and increase tuber deformity, due to its phytotoxic properties (2). Therefore for 2,4-D to become a practical disease control method, these negative side effects need to be addressed. Initial studies indicated that application rates could be reduced, while still providing resistance to common scab. Optimal timing and reduced spray frequencies could lead to further rate reductions.

MATERIALS AND METHODS

Plant establishment and treatments Potato plants of two varieties (Russet Burbank and Desiree) were grown from seed in pots filled with soil that had been inoculated with *S. scabiei*. Sprays of 2,4-D were applied to the foliage of the plants at various rates, frequencies and times. In addition to pot trials, two field trials were undertaken in different locations with histories of common scab in prior potato crops.

Disease assessment and toxin tolerance After harvest, lesions on tubers were scored for coverage (incidence) and depth (severity) (1). Toxic resistance was assessed by placing disks of filter paper soaked in thaxtomin A upon the sterile cut surface of tuber slices. Slices were kept in the dark for 7 days and the severity of the necrosis under the filter paper disks was scored. Those with less necrosis were deemed to have greater tolerance to the toxin than those with more necrosis.

RESULTS AND DISCUSSION

The lowest rate reduced disease incidence to an equivalent level to all other rates, suggesting that only a small amount of 2,4-D is required within the tubers to induce resistance (see Figure 1).

Additionally, the results obtained from the 2008/09 trials further suggested that treatment timing was an important factor in controlling common scab through foliar applications of 2,4-D. Those plants sprayed at 10 and 20 days after emergence (DAE) had significantly less disease than those sprayed at 30DAE and later (see Figure 2).

Tubers internodes are susceptible to infection by *S. scabiei* 1 to 2½ weeks after formation (3). As these 10 and 20DAE sprays were applied prior to, or during this 'infection window', they provided protection to the tubers, while later sprays provided significantly less protection. This suggests that for 2,4-D to induce resistance in tubers to common scab, the 2,4-D must be present in the tubers at the time of infection, although later sprays may provide protection to tuber parts that are still expanding.

![Figure 1](image1.png)

**Figure 1.** Disease incidence scores for Russet Burbank and Desiree combined, for single sprays applied at the given rates. Data is from the 2008/09 pot trials.

![Figure 2](image2.png)

**Figure 2.** Disease incidence scores for Russet Burbank, for single sprays of 100mg/L 2,4-D applied between 10 and 60DAE. Data is from the 2008/09 field trial.

ACKNOWLEDGEMENTS

This work was facilitated by Horticulture Australia Limited (HAL) in partnership with the Potato Processing Association of Australia, and was funded by the potato levy. The Australian Government provides matching funding for all HAL’s R&D activities.

REFERENCES

INTRODUCTION
Polymyxa graminis is a plasmodiophorid that is an obligate, biotrophic parasite of the roots of graminaceous plants. It is economically important as the vector of several soil-borne virus diseases of cereals in other countries (1). None of these virus diseases has been recorded in Australia. *P. graminis* has been recorded on the grass *Poa annua* at Murrumburra in NSW in 1959 (2) but not on cereals.

As part of research on the role of zoosporic parasites in cereal crops (3), poorly growing barley in a farmer’s field near Wondai was investigated and *P. graminis* was detected by microscopy and PCR of ribosomal DNA with species specific primers.

MATERIALS AND METHODS
Collection of plant samples Symptomatic and asymptomatic barley cv. Dictator plants were excavated to 20 cm depth at 3 positions in the field and kept in a cold room at 3°C pending analysis.

Plant tops and roots The number of tillers, and dry masses of tops and roots per plant were determined for symptomatic and asymptomatic plants.

Root Microscopy Roots were extracted from 250-g subsamples of soil and cleaned by repeated washing on a 425 µm sieve. A 0.5 g subsample of roots was cleared with KOH and stained with 0.1% trypan blue in lactic acid. Roots were examined under a stereo microscope and quantified for Polymyxa sporosori using a grid intersect method. Selected root pieces were mounted on microscope slides and examined at magnifications up to x400 under a compound microscope.

PCR Analysis Separate subsamples of 50 mg roots were freeze-dried, ground in a mixer mill, and extracted for DNA using a modified CTAB extraction method, then re-extracted using a QIAamp DNA Stool Mini Kit (Qiagen #51504). Modified PCR protocols (4) for the Polymyxa genus specific (Psfwd1/Pxrev7) and *P. graminis* types I and II-specific (Pgfwd2/Pxrev7) primer sets were used, and PCR products sized by electrophoresis in 4% agarose gels with ethidium bromide staining.

Electron microscopy Leaf dip preparations were negatively stained with 1% ammonium molybdate and examined in an electron microscope.

RESULTS
Barley plants When observed 3 months after sowing, symptomatic barley plants were stunted (about 20 cm high) with chlorosis of the distal portions of the lower leaves appearing like nitrogen deficiency. Asymptomatic plants were about 60 cm high, phenologically more advanced and non-chlorotic. Symptomatic plants occurred in patches in one corner of the field and along tractor wheel track lines. A plough pan was noted when digging samples. Symptomatic plants were only 0.56 and 0.45 the top weight and root weight, but 2.4 times the tiller number, respectively of asymptomatic plants. Sporosori of *P. graminis* (Fig. 1) were present in the roots of both symptomatic (12.2% root intersects with sporosori) and asymptomatic plants (3.8% with sporosori).

Figure 1. Sporosori of *Polymyxa graminis* in a rootlet of barley from a field near Wondai, Qld.

PCR Analysis A PCR product of 320 bp with both primer sets confirmed the presence of *P. graminis* type II (syn. *P. graminis* f. sp. tepida) in the roots of both symptomatic and asymptomatic plants (4).

DISCUSSION
This study clearly demonstrates the presence of *P. graminis* in an Australian barley crop. No virus was detected in the leaves. The symptoms might result from soil compaction and nitrogen deficiency. *P. graminis* type II is most commonly found on wheat overseas and is hosted by both barley and oats. The presence of *P. graminis* in fields increases the potential for the establishment and spread of a soil-borne virus should any gain entry to the country. A survey for the presence of *P. graminis* in fields used for grain growing in Australia is needed to assess this potential.

ACKNOWLEDGEMENTS
We thank GRDC for financial support.

REFERENCES

POLYMYXA GRAMINIS IN A CEREAL CROP IN AUSTRALIA

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6th Australasian Soilborne Diseases Symposium, 2010 97
GENETIC RESISTANCE IN WHEAT TO ROOT-LESION NEMATODE
(PRATYLENCHUS THORNEI)

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INTRODUCTION

Root-lesion nematodes (P. thornei and P. neglectus) are a serious threat to wheat production in the northern grain region of Australia with an annual loss of $69 M/year (1). P. thornei occurs more frequently and at higher population densities than P. neglectus in the northern region (2). Targeted wheat breeding has produced wheat cultivars with improved levels of tolerance (yielding capacity under nematode attack) to P. thornei (1). However, genetic resistance to nematode multiplication is urgently required in commercial cultivars. This paper reviews available information on resistance to P. thornei in the northern region.

MATERIALS AND METHODS

Field experiment A field experiment was conducted at Formartin on land infested with P. thornei in which various wheat cultivars were cropped in the same plots for 3 successive years and compared with plots kept in clean fallow. Soil was sampled in intervals to 90 cm depth and P. thornei extracted in Whitehead trays and counted in Hawksley slides under a microscope.

Glasshouse resistance test Initial experiments were conducted to ‘calibrate’ glasshouse tests for cultivar resistance with the field resistance results. Subsequent research refined the methods to achieve optimum conditions for P. thornei reproduction limited only by the level of genetic resistance in the cultivar/line. Currently, single plants are grown in pots of 330 g pasteurised vertisol with 10000 kg soil and 1 g Osmocote fertiliser, single plants are grown in pots of 330 g pasteurised vertisol and replicates are grown in one pot. The pot is watered at the rate of 15 ml per week for 16 weeks. The soil is excavated from each pot and the number of nematodes in the soil examined using a Hawksley slide.

Sources of resistance The glasshouse resistance test has been used to search for sources of resistance to P. thornei in various collections of Aegilops tauschii (the D genome donor to wheat) (3), durum wheat (AB genomes), and landrace wheats (ABD genomes) from West Asia and North African (WANA) countries (4) including a comprehensive collection from Iran (5). Inheritance of resistance to P. thornei in wheat and quantitative trait loci associated with resistance has also been investigated using the glasshouse methods.

RESULTS AND DISCUSSION

Field Resistance In the field experiment, GS50a (a reselection from cv. Gatcher) was found to have a useful level of resistance to P. thornei (Fig. 1).

Glasshouse resistance tests Resistance to P. thornei at a similar or greater level to that in GS50a has been found in a number of accessions of Ae. tauschii (3), durum wheat (3), synthetic hexaploid wheat (3) and wheat landraces (4, 5). Inheritance of resistance was found to be polygenic and additive (6, 7) with QTL on all three genomes. Breeding resistant cultivars using available germplasm and screening progeny for resistance in glasshouse tests is possible and would be of immense value to northern grain growers. The availability of resistant cultivars would (a) support tolerance in reducing loss in the current crop (b) reduce nematode populations residual in soil to attack subsequent crops and (c) reduce the rate of spread of P. thornei to new fields and subregions.

REFERENCES

QUANTIFYING TUBER- AND SOIL-BORNE INOCULUM OF RHIZOCTONIA SOLANI IN POTATO PRODUCTION SYSTEMS IN NEW ZEALAND

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INTRODUCTION
Rhizoctonia solani causes delayed emergence and stand reduction (stem canker) of potato, and sclerotia on tubers (black scurf). These diseases are key production issues limiting performance of New Zealand’s potato industry.

The relationship between inoculum and disease development is poorly understood for R. solani, and the relative importance of soil- and tuber-borne inoculum remains unknown. This is complicated by the ability of more than one anastomosis group (AG) of the pathogen to infect potato (predominantly AG-3 and AG-2-1). Different AGs have unique ecological niches, and each can cause distinct disease symptoms (1).

DNA monitoring tools have been developed to quantify AGs on potato overseas (2, 3). Here, we report evaluation of these tools for the detection of AGs on tubers and in soils from production systems in New Zealand. We also describe the use of these tools to examine the effect of cropping regimes on inoculum of key R. solani AGs on tubers and in soil.

MATERIALS AND METHODS
DNA extraction from tubers Sap was extracted from the skin peeled from each tuber. DNA was extracted by bead-beating a 500 µL aliquot of the sap in a 2 ml screw-cap tube containing tris-based extraction buffer and 1 g of steel beads. DNA was then purified using a silica-based protocol.

DNA extraction from soils Triplicate post-harvest soil samples (each consisting of ten soil cores) were collected randomly from each plot in a field rotation trial. Sub-samples (50 g) were then dried, and the DNA was extracted using a tris-based extraction protocol.

Quantitative Taqman real-time PCR (qPCR) assays qPCR assays for AG-3 and AG-2-1 (2) were performed on DNA extracted from tubers or soil. All DNA samples were also tested for the presence of inhibitors using internal positive controls.

RESULTS
qPCR assays were evaluated by comparing AG-3 and AG-2-1 DNA levels on 20 tubers certified as “clean” and 20 tubers rejected because of black scurf by the NZ Seed Certification Authority after visual inspection. The assays showed that levels of AG-3 DNA were greater on rejected tubers, whereas levels of AG-2-1 DNA were greater on certified tubers (Fig. 1).

The incidence of black scurf on tubers was generally greatest in plots with the greatest amounts of AG-3 DNA on tubers and in soil (data not shown).

DISCUSSION
DNA monitoring tools enabled the quantification of AG-3 and AG-2-1 DNA from tubers and soils in potato production systems in New Zealand. AG-3 and AG-2-1 was detected on tubers without visible disease symptoms. The role of this inoculum in the spread of R. solani remains unknown. This technology is presently being evaluated as a disease prediction tool for growers.

ACKNOWLEDGEMENTS
This research was funded by the New Zealand Foundation for Research, Science & Technology (Contract LINX0804).

REFERENCES

Figure 1: AG-3 and AG-2-1 DNA on certified and rejected tubers.

To examine the impact of different cropping regimes on R. solani AG-3 and AG-2-1, tubers and soils were collected from a rotation trial comprising 24 plots (four replicates of six crop rotations) located at Pukekohe, New Zealand (4). Cropping regimes influenced R. solani DNA levels both on tubers and in soil. For example, AG-3 DNA levels on tubers and in soil samples collected from plots where potato crops had grown continuously for 5 years (Plots 3, 9, 16 & 22 (unshaded)) were generally greater than from plots with a 5-year rotation of potato, potato, onion, onion, potato (Plots 6, 7, 18 & 21 (black)) (Figs 2 & 3).

Figure 2: AG-3 DNA from five tubers collected from each plot.

Figure 3: AG-3 DNA from soils collected from each trial plot.
THE ROLE OF ROTATION CROPS IN MANAGING PLANT-PARASITIC NEMATODES ON GINGER IN FIJI

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INTRODUCTION

In Fiji, two important nematode pests occur on ginger (Zingiber officinale), namely root-knot nematode (Meloidogyne spp.) and burrowing nematode (Radopholus similis). Reniform nematode (Rotylenchulus reniformis) is probably also a pathogen, but its pest status has never been determined. It commonly occurs at high population densities on ginger and is a recognised pest of other tropical crops. This paper examines the role of rotation crops in managing these three nematode species.

MATERIALS AND METHODS

Initial and final nematode population densities (P\textsubscript{i} and P\textsubscript{f}, respectively) were determined in typical ginger-growing fields in the Navua, Veikoba and Weibau regions. Ginger, and the crops that usually follow it in the rotation (cassava and taro), were sampled at the time each crop was planted and harvested. Soil (200 mL) was spread on a Whitehead tray and nematodes were retrieved on a 38 µm sieve after 2 days. Multiplication rates for particular nematodes were determined as Pf/Pi.

Multiplication rates for R. similis on taro and cassava were determined in 1 L pots of pasteurised sand and peat. Pots were inoculated with 1,000 R. similis and 40 weeks later, nematodes were extracted from soil (as above) and from roots that were macerated in a blender and then spread on an extraction tray.

RESULTS

R. reniformis was the most common plant-parasitic nematode in ginger-growing soils. It multiplied readily on ginger, whereas there was little multiplication on taro or cassava (Table 1).

<table>
<thead>
<tr>
<th>Crop</th>
<th>Nematodes/200 mL soil</th>
<th>P\textsubscript{i}</th>
<th>P\textsubscript{f}</th>
<th>P\textsubscript{f}/P\textsubscript{i}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature ginger</td>
<td>93 2410 25.9</td>
<td>30 710 23.7</td>
<td>540 7600 14.1</td>
<td></td>
</tr>
<tr>
<td>Mature ginger</td>
<td>805 3550 4.4</td>
<td>370 4560 12.3</td>
<td>40 830 20.8</td>
<td></td>
</tr>
<tr>
<td>Taro</td>
<td>1535 350 0.2</td>
<td>90 104 1.2</td>
<td>2440 1328 0.5</td>
<td></td>
</tr>
<tr>
<td>Cassava</td>
<td>390 280 0.7</td>
<td>436 130 0.3</td>
<td>490 380 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1120 1560 1.4</td>
<td>1010 380 0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

In the Fijian ginger farming system, ginger is generally grown every 3 years, with crops of cassava and taro planted in the years between ginger crops. Our results indicate that this is an excellent rotation for managing the three plant-parasitic nematodes likely to cause damage on ginger. Taro and cassava are poor hosts of both R. reniformis and R. similis, while Meloidogyne spp. rarely reaches high population densities in ginger-growing soils, regardless of the crop that is grown.

Options for improving nematode management in ginger include immersing 'seed' pieces in hot water to eliminate nematodes from planting material, removing volunteer ginger plants from crops that follow ginger, and controlling weeds known to host burrowing nematode (e.g. crowsfoot, Eleusine indica).

ACKNOWLEDGEMENTS

Funding from ACIAR is gratefully acknowledged.
INTRODUCTION
French, dwarf, runner or climbing beans are all green beans (Phaseolus vulgaris L.) and they are valuable crops to Australia. Beans are grown for fresh market and for processing (i.e. canned or frozen).

Beans are susceptible to an array of soil-borne pathogens including Aphanomyces euteiches, Thielaviopsis basicola, Macrophomina phaseolina, Rhizoctonia solani, and species of Pythium and Fusarium. They can be associated with bean root disease singly or in combinations, and often referred to as a “complex”. Aphanomyces root rot (ARR) has been identified as an issue in the Valla region of New South Wales (NSW) (1). It is particularly severe when beans are grown regularly on the same block. In Australia, *Aphanomyces* has been recorded on other crops, and members of the genus can also cause diseases of fish.

A thorough review of *Aphanomyces* species affecting peas and sugar beet was undertaken (2). Since that review, the fungus has been identified on beans associated with root rot (3, 1).

*Aphanomyces* is very difficult to isolate from plant material. A selective media for *Aphanomyces* species is available but it is not always successful at isolating the fungus alone. Identifying the fungus on the plant may be achieved by placing roots in Petri dishes with water and watching under a microscope over the next 48 hours for the characteristic sporangia and the method of zoospore production. However some isolates can be difficult to induce to produce zoospores. As a result of ongoing occurrences of ARR on beans, the disease was investigated.

MATERIALS AND METHODS
Pathogenicity An *Aphanomyces* species was isolated from infected plants collected in Valla and used in a pathogenicity test. Sterile vermiculite was placed in ten 100 mm pots and five bean seeds (“Simba” treated with metalaxyl) were planted in each. An isolate of one-week-old culture of *Aphanomyces* grown on 1/4 strength Potato Dextrose Agar (PDA) was inoculated onto five-day-old bean plants by mixing the contents of half of a 90 mm culture plate into the vermiculite. Plants were maintained with sterile water. In order to induce infection, inoculated plants were watered three times a day for three days from three days after inoculation. After a further two weeks, plants were assessed by examining hypocotyls for ARR lesions. Controls pots were inoculated with PDA without *Aphanomyces*.

Effect of bean history on disease expression Twelve soils with varying histories of bean growing were collected from growers’ properties and nearby blocks. Each soil was placed into totes (square plastic containers with dimension 385 mm long x 290 mm wide x 130 mm deep, with soil depth 75 mm). Forty seeds (as used above) were sown into each tote, there were six replicates per soil. Plants were maintained in a glasshouse at 20 and 30°C. At the two-leaf stage, plants were watered three times a day to induce *Aphanomyces* infection. Twenty two days after sowing, plants were assessed by counting the number with typical ARR hypocotyl lesions as a percentage of the total that had germinated in each tote.

RESULTS
Pathogenicity Bean plants that were inoculated with *Aphanomyces* showed clear symptoms compared to those in control pots. Symptoms consisted of browning of the hypocotyl region and of the roots. *Aphanomyces euteiches* was re-isolated from infected plants.

Effect of bean history on disease expression The disease was more severe in soils that were more recently cropped with beans (Table 1). However, soils that had not had beans for three to ten years previously had high levels of infected plants.

Table 1. Effect of time since beans on disease severity

<table>
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<th>Soil Farm</th>
<th>Years since last bean crop</th>
<th>Percentage* infection</th>
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<tr>
<td>1 1</td>
<td>30</td>
<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 2</td>
<td>Never</td>
<td>6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 3</td>
<td>6</td>
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<td>28&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>12 3</td>
<td>0</td>
<td>96&lt;sup&gt;g&lt;/sup&gt;</td>
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* Values with the same letter are not significantly different at the 1% level of significance.

DISCUSSION
*Aphanomyces euteiches* causes browning of roots and hypocotyls and severely affects the growth of green bean plants. The long term survival of the fungus in soil has implications on replanting beans after beans. Disease is likely where fresh land is not available. Control of this disease that is difficult to detect is a high priority.

ACKNOWLEDGEMENTS
Part of this work was funded by Horticulture Australia Ltd.

REFERENCES
INVESTIGATING THE ROLES OF RHIZOCTONIA SOLANI AG2.1 AND 3 IN CAUSING STOLON PRUNING AND STEM CANKER IN POTATOES

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INTRODUCTION

Rhizoctonia solani causes stem and stolon canker on potato plants, as well as black scurf on potato tubers. AG2.1 and AG3 are the most dominant anastomosis groups isolated from potato plants (1). AG2.1 is prevalent as mycelium in field soils that grow potatoes. AG3 is less prevalent in potato field soils and survives as melanised sclerotia (2). The interactions between AG2.1 and 3 have not been fully examined. Our previous study, using radish as a model system, found AG2.1 and AG3 act synergistically to produce disease (3). This present study used potato to investigate whether AG2.1 and AG3 compete with each other in soil, or act synergistically to produce disease.

MATERIALS AND METHODS

Soil inoculation An isolate of AG2.1 and AG3 (both originally from potato) were inoculated into soil in combination at various rates: one plate fungal mycelium (1); half plate fungal mycelium (0.5); quarter plate fungal mycelium (0.25) per 8 kg soil (Table 1). One cv, Coliban minituber was planted per pot with 5 replicate pots, and grown in the glasshouse. Emergence was assessed 4 weeks after planting. Stolon pruning and stem canker lesions were assessed at 4 times at 3-weekly intervals, beginning at 37 days after planting.

Table 1. Soil inoculation combinations and rates (plates/8kg soil).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AG2.1</th>
<th>AG3</th>
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<tr>
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<td>0.25</td>
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<tr>
<td>12</td>
<td>0</td>
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</table>

RESULTS AND DISCUSSION

Stolon pruning By itself, AG2.1 produced only minor stolon pruning symptoms at each sampling date (Figure 1), but AG3 by itself caused 32-58% stolon pruning over the 4 sampling dates. When AG2.1 and AG3 were combined at the highest rate (treatment 3), stolon pruning symptoms increased from 22% to 53% over the sampling dates.

Stem canker Only the highest rate at the last sampling date of AG2.1 alone produced stem cankers (14% stems infected) (Fig. 2). In contrast, AG3 by itself produced 5% stem cankers by the first sampling date, increasing to 90% by the second sampling date.

The rate of AG3 starting inoculum had no effect on the level of stolon pruning and stem canker, with the lowest rate causing as much disease as the highest rate. These results suggest that on potato, unlike radish, AG2.1 and AG3 do not act synergistically to produce disease symptoms. Future research will use these treatments and growing conditions to monitor soil colonisation overtime and study disease expression in potato plants.

REFERENCES

COLLECTION AND IDENTIFICATION OF TRICHODERMA SPECIES IN GREEN SPACES OF TEHRAN

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INTRODUCTION
Species of the genus Trichoderma are cosmopolitan and typically soil-borne or wood-decaying fungi. They frequently dominate components of the soil micro flora. Some species are economically important and produce industrial enzymes and various antibiotics like alamethycins. Furthermore, these fungi are involved in biological control of plant pathogens and also benefit plant health and nutrient uptake. So far, 13 species of Trichoderma have been identified from Iran (1, 3, 4, 5, 6).

MATERIALS AND METHODS
In this study, soil from different parks in various places in Tehran was sampled. One hundred and seventy Trichoderma isolates were collected and various morphological characteristics were studied, namely colony radius in different media and at various temperatures, the morphology and size of conidia, conidiophores, phialides, chlamydospores, aerial mycelia and submerged mycelia (1, 2, 5).

RESULTS AND DISCUSSION
On the basis of the above morphological characteristics, these isolates were classified within three sections of the genus Trichoderma. Ten isolates belonged to Longibrachitum section including T. citriniviride, T. ghanense, T. brevicompactum, T. polysporum and Trichoderma sp. Another 135 isolates belonged to the Trichoderma section (4). A further 25 isolates were placed in Pachybasium section, including T. hamatum, T. polysporum and Trichoderma sp. The most frequent species was T. harzianum.

Of the species recovered, T. harzianum and Trichoderma sp. from Pachybasium section are new records for Iran.

REFERENCES
INTERACTION OF VERTICILLIUM DAHLIAE AND MELOIDOGYNE JAVANICA IN SENSITIVE AND RESISTANT OLIVE SEEDLINGS

INTRODUCTION

Root-knot nematode (Meloidogyne javanica) causes considerable damage to olive groves in Iran, while the causal agent of verticillium wilt (Verticillium dahliae) limits production in leading olive-producing countries (3). Interactions between fungal and nematode pathogens have been studied in different hosts by investigators around the world (5) and this study looked at the impact of V. dahliae and M. javanica on defensive mechanisms (1) in olive cultivars.

MATERIALS AND METHODS

The non–defoliант strain of V. dahliae (SS-4) was isolated from olives showing disease symptoms and propagated on tomato cv. Rutgers (4). Second-stage juveniles (J2) and conidia and microsclerotia were used as sources of inoculum of the nematode and fungus, respectively. One – year-old seedlings of olive cultivars Zard, Rognani, Koroneiki and Manzanilla were transplanted into pots containing 2000 g of sterilized sandy loam soil. The experiment was a completely randomized design with 32 treatments and 5 replicates. Pots were placed on glasshouse benches at a temperature of 25–27 °C and plants received natural light (6, 8, 11, 15). Treatments were a control, nematode alone, fungus alone and fungus + nematode. Pots were inoculated with 2000, 3000 or 4000 J2 and /or 10 microsclerotia /g soil, depending on the treatment.

RESULTS

The presence of the nematode caused a reduction in colonization by the fungus in the root and stem. Similarly, the presence of the fungus reduced the number of galls and egg masses produced by the nematode. Severe fungus wilt was observed on aerial parts of cultivar Zard when both pathogens were inoculated, while mild fungus wilt was observed in the fungus alone treatment of cultivar Koroneiki. The fungus reduced galling and egg mass production on cvs. Manzanilla , Zard , Rognani and Koroneiki, (p≤0.05) (3,12,13).

The concentration of phenolic compounds and total proteins, soluble peroxidase (SPOX), ionically cell –wall-bound peroxidase (CWPPOX) (10, 11), phenylalanine aminohalase (PAL), polyphenol oxidase (PPO), catalase (CAT), β-1, 3-glucanase and β-1, 4-glucanase were studied in roots and leaves 1, 10, 20 and 30 days after inoculation. The experiment was terminated after 10 months and the following parameters were measured: seedling height; fresh weights of root s and stems; number of galls and egg masses per root system; percentage incidence of colonization by the fungus.

DISCUSSION

Based on results obtained in this study, the content of phenolic compounds and total proteins and the quantitative activity of enzymes associated with resistance (PPO, PAL,CAT, COWPOX, β-1 3-glucanase and β-1 4-glucanase) in roots and leaves varied with cultivar. It was greatest in cv. Koroneiki and significantly lower in Rognani, Zard and Manzanilla.

REFERENCES

INTRODUCTION

*Pythium* spp. are associated with pre- and post-emergence damping-off in seedlings and cavity spot of various Apiaceae vegetable crops and often occur in complexes with other pathogens. They can also reduce plant productivity by attacking root hairs and lateral roots, impairing water and nutrient uptake, thus earning the reputation of a ‘common cold’ of plants, because this damage often goes unnoticed (1).

Preliminary research has implicated *Pythium* spp. as one of the possible causes of parsnip canker in Victoria (2), particularly in crops seeded in February and grown over winter period for harvest in October. Victoria produces 80% of the Australian parsnip crop. The highly marketable white-fleshed parsnip is more susceptible to canker than the less marketable, yellow-fleshed parsnip.

Field trials were conducted on sites with different soil textures (sandy loam and medium clay) with the objective of determining the role of *Pythium* spp. in the development of parsnip canker and to identify potential disease management options.

MATERIALS AND METHODS

Field trials Trials were laid out as randomised complete block designs with eight replications of seven treatments on the sandy site (Clyde, Victoria) and six replications of six treatments on the clay site (Devon Meadows, Victoria). Treatments on both sites were (i) untreated control; (ii) azoxystrobin (Amistar® 250 SC) applied at weeks 8, 15 and 21 after seedling emergence; (iii) metalaxyl (Ridomil Gold® 25G) applied at weeks 1 and 8; (iv) metalaxyl at week 8; (v) metalaxyl at weeks 15 and 21; and (vi) metalaxyl at weeks 1, 8, 15 and 21.

*Streptomyces lydicus* (Microplus™) was applied 6 times at monthly intervals to the trial on the sandy loam site. Trials were direct seeded (four rows per bed) with the growers’ own seed on 8 April 2009 and crops were maintained by the growers. Parsnips were harvested on 21 and 29 October 2009, at Clyde and Devon Meadows, respectively.

**Assessment** Incidence and severity of parsnip canker were assessed at harvest with the proportion of unmarketable parsnips expressed as the percentage of parsnip roots with disease and other damage (skin cracks, forking). Disease severity was calculated for each sampled parsnip using a scale of 0 to 4 where 0 = healthy root; 1 = superficial brown lesions on the upper tap root or lesion on the lower tap root; 2 = elongated lesion on the tap root; 3 = a deep lesion or canker on the tap root; and 4 = crown rot. Data were analysed using ANOVA.

RESULTS

On the sandy loam site, all metalaxyl treatments, irrespective of the time and number of applications, reduced the incidence of parsnips with canker and increased marketable yields by approximately 30%. None of the metalaxyl treatments reduced the incidence of canker on the medium clay site (Fig 1). Neither the *S. lydicus* nor the azoxystrobin reduced the incidence of canker or improved yields at either site, but they did reduce the severity of canker on the sandy loam site (Fig 1, Table 1).

DISCUSSION

The reduced incidence of parsnip canker following the metalaxyl treatments at the sandy loam site suggests an association between oomycete pathogens and damage to the parsnip roots. *Pythium* spp. were commonly isolated from young parsnip roots sampled at monthly intervals from both trial sites (Petkowski *et al*., unpublished). The reason for the lack of control of parsnip canker with metalaxyl at the medium clay site is not known, although enhanced degradation of the fungicide could be a possibility. Further research is required to determine the role of *Pythium* spp. in the development of parsnip canker.

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REFERENCES

INDEX OF AUTHORS

Aalders LT 33 Evans ML 45
Agarwal A 65 Exell GK 56
Aitken EAB 58, 62, 68 Falloon RE 34, 41, 46, 48
Akinsanmi OA 28 Forsyth LM 47
Allen D 43 Furlong MJ 68
Anstis ST 29 Garland-Campbell K 76
Backhouse D 30, 31 Gau RD 48
Badi A 32 Geense PF 47
Beardsell SV 42 Ghasemi A 82
Behboudi K 82 Guijarro MB 49
Bell MJ 5, 88 Guilhabert M 67
Bell NL 33 Gupta VVAR 50, 51
Benger RW 53 Halpin NV 88
Berry GW 70 Hamill SD 52, 89
Bienkowski D 34 Hampton JG 35, 60
Bithell SL 36, 37 Harding R 53
Blumenthal MJ 13 Harper S 5
Bolat N 17, 69 Hay A 59
Braithwaite M 34, 54, 59, 60 Herde DJ 61, 72
Braun HJ 17 Herdina 53
Brett RW 49 Hicks E 34, 54
Brown PH 95 Hollaway GJ 55, 56
Browne L 101 Imsic M 43
Brunner PC 48 Jaspers MV 57
Butler RC 37, 41, 46 Jennings R 97
Cahill MJ 65 Jones EE 57
Chandolu V 43 Jones KW 58
Chohan OJ 35 Jones RB 43
Clewett TG 71, 97, 98 Kandula DRW 35, 60
Cobon JA 68, 70 Kandula J 59
Cochran A 39 Keenan S 99
Coleman DC 1 Khatri BB 95
Cook A 51 Kheiri A 104
Cribb B 58 Kiarudi M 102
Cromey MG 36, 37 Kilinc AC 69
Crump NS 38 Kinaci E 44
Crump NS 41, 46 Kirkegaard J 51
Curtin D 36, 37, 46 Klix M 39
Dababat AA 39, 69 Knight NL 61, 74
Dann EK 49, 86 Kukulies, T 47
Das, S 41 Lawrie AC 32
de Boer RF 42, 73, 105 Le PD 62
Diallo S 51 Lego S 67
Dick MA 83 Lehmensiek A 61
Donald EC 32, 43, 65, 83 Li Y 63, 64
Dore DS 57 ListerRA 46
Drenth A 28 Loguercio L 34
Eastwood R 8 Long D 67
Edwards JE 20, 49 Lovelock D 65
Elekioglu IH 69 Male MF 65
Erginbas G 44, 69 Manes Y 24
Manker DC 67  Sari E 81
Marshall D 38  Scoble CA 43, 84
Martinez E 67  Scott CL 46
Mathews K 24  Shah FA 41
Mattner SW 20, 43, 49  Sharifi-Tehrani A 82
McCarthy JW 68  Sheedy JG 85, 97, 98
McDonald BA 48  Sikora RA 39
McGee PA 10  Silva D 67
McKay AC 11, 36, 37, 51, 53, 55, 56  Smiley RW 76
Mele PM 13, 15  Smith D 51
Merz U 48  Smith EKA 49
Minchinton EJ 73, 105  Smith JP 87, 89
Molina AB 47  Smith LA 87
Nejad-nasrolah F 79  Smith MK 62, 87, 89, 90, 100
Nelson T 99  Snudden M 101
Nicol JM 17, 24, 44, 69  Spiers TM 83
Noble R 2  Stewart A 22, 34, 35, 54, 59, 60
Northcott GT 83  Stirling AM 90
O’Brien PA 19  Stirling GR 63, 64, 87, 88, 89, 90, 100
O’Neill WT 70  Sunkad G 91
Ophel-Keller K 11, 51, 53  Sutherland MW 61, 74
Orchard B 78  Taheri P 92, 93, 94
Owen KJ 71, 97  Tarighi S 92, 93, 94
Pan M 54  Taylor JT 83
Pattison AB 5, 47, 70  Tegg RS 95, 96
Paulitz TC 76  Thompson HK 96
Pederick SJ 29  Thompson JP 71, 97, 98
Pegg KG 40, 86  Thompson SE 99
Percy CD 72  Thompson F 42, 73, 105
Persley DM 97  Tiedje JM 4
Petkowski JE 73, 105  Todd C 53
Petrisko JE 74  Toktay H 69
Putman AR 41, 99  Trethowan R 24
Pittaway PA 75  Turaganivalu U 100
Plummer KM 84  Van Zwieten L 25
Poole GJ 76  Vawdrey LL 66
Porter IJ 20, 43, 49, 65, 77, 84  Verstraten J 102
Purdue AV 55  Villalta ON 43
Rahman L 78  Walgenbach P 67
Rahnama K 79  Wallwork H 27, 45
Rames E 52  Walters TL 72
Ranjarb N 82  Wardynski M 102
Rawsley B 80  Warkentin D 67
Razavi M 81, 82  Washington WS 42
Reddy NPE 50  Watrin C 39
Reen RA 85  Watson A 101
Reglinski T 83  Weckert M 78
Richardson F 102  Whiley AW 86
Riches D 43  Wicks TJ 29
Ridgway HJ 57  Wiechel TJ 49, 102
Roget DK 51  Wilson CR 95, 96
Saeedizadeh A 104  Wite D 43
Sahin E 69  Wright PJ 99
<table>
<thead>
<tr>
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**4th Australasian Soilborne Diseases Symposium**
**Queensland, Australia**
**9 - 11 August 2010**

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