

APPS 2009

Plant Health Management: An Integrated Approach

29 September – 1 October 2009
Newcastle City Hall

ISBN 978-0-646-52919-6



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Welcome

On behalf of the Local Organising Committee welcome to Newcastle and the 17th Australasian Plant Pathology Society Conference, an event that marks the 40th (or Ruby) anniversary of the Australasian Plant Pathology Society. It provides us with a good opportunity to reflect on the achievements of our profession over four decades of unprecedented discovery about the nature and management of plant disease. It is also a time to ponder the directions of our profession amidst the challenges posed by emerging and persistent plant diseases, food security, climate change, water shortages, rising atmospheric carbon dioxide levels, bioterrorism, consumer safety and preferences, and the opportunities presented to agriculture and horticulture by biofuels, phytomedicines and leisure activities.

The conference theme 'Plant Health Management: an integrated approach' addresses these challenges from three angles—fundamental discovery, the application of these discoveries to practical problems and the adoption of research. Local and international keynote speakers have been invited to challenge you with their perspectives on the big questions in plant pathology. Many of you will have already been challenged by, and enjoyed, the supporting program of workshops and field trips.

Newcastle is a bustling, historic, post-industrial seaside city boasting exciting cultural activities, superb beaches, and other nearby attractions including the Hunter Valley, Barrington Tops National Park and more superb coastal scenery. Please take time to enjoy the location, catch up with friends and colleagues, meet new ones, and return home invigorated, wiser and happy.

David Guest

Conference Convenor, APPS 2009

Conference Organising Committee

- David Guest, Convenor
- Rosalie Daniel
- Robert Park
- Peter Magee
- Nerida Donovan
- Len Tesoriero
- Angus Carnegie
- Chris Steel
- Gavin Ash

Workshop Convenors

Microbial ecology—concepts and techniques for disease control
—Kerry Everett

Tree Pathology Workshop
—André Drenth and Angus Carnegie

Magical Mystery Vegetable Tour
—Len Tesoriero and Nerida Donovan

Biology and management of organisms associated with bunch rot diseases of grapes—Chris Steel

Conference Secretariat

Conference Logistics*
PO Box 6150
Kingston ACT 2604
















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conference@conlog.com.au
www.apps2009.org.au

*acting as agent for APPS



Sponsors

The Local Organising Committee gratefully acknowledges the support of our sponsors:

Conference sponsors	Grains Research and Development Corporation	 Grains Research & Development Corporation	
	HAL	 <i>Know-how for Horticulture™</i>	
Gold sponsor	APPS		
Silver sponsor	Nufarm Australia and BASF		 The Chemical Company
Welcome Reception	Cooperative Research Centre for National Plant Biosecurity		
International speaker and post-conference tour sponsor	Grape and Wine Research and Development Corporation		
Keynote speaker	Forest and Wood Products Australia Limited		
Lunch, Day 2	Agrichem		
Supporters	Plant Health Australia		Lomb Scientific
			
	AusVeg		The Crawford Fund
			
	Mars		The University of Sydney
			

Exhibitors

APPS



The Australasian Plant Pathology Society is dedicated to the advancement and dissemination of knowledge of plant pathology and its practice in Australasia. Australasia is interpreted in the broadest sense to include not only Australia, New Zealand and Papua New Guinea, but also the Indian, Pacific and Asian regions. Although the Society's activities are mainly focused on the Australasian region, many of the activities of our members are of international importance and significance.

The Society was founded in 1969. Our members represent a broad range of scientific interests, including research scientists, teachers, students, extension professionals, administrators, industry and pest management personnel.

FOR MORE INFORMATION:

Dr Peter Williamson
Business Manager
APPS Inc

Telephone (07) 4632 0467
Facsimile (07) 46378326
www.appsnet.org

Leica Microsystems



Leica Microsystems is a leading global designer and producer of innovative high-tech precision optics systems for the analysis of microstructures.

It comprises 11 manufacturing facilities in eight countries, sales and service companies in 20 countries and an international network of dealers; the company is also represented in over 100 countries and the international headquarters are based in Wetzlar, Germany.

Leica Microsystems is one of the market leaders in each of the fields of microscopy, confocal laser scanning microscopy, microscope software, specimen preparation and medical equipment. The company manufactures a broad range of products for numerous applications requiring microscopic imaging, measurement and analysis. It also offers system solutions in the areas of life science, including biotechnology and medicine, as well as the science of raw materials and industrial quality assurance.

Specific to this conference, we will be displaying automated compound and stereoscopic microscopes highlighting our imaging systems using Montage, multifocus 3D imaging and Web Module allowing viewing and analysis of images from a remote station via internet.

FOR MORE INFORMATION:

1800 625 286 [ph]
www.leica-microsystems.com

Leica Microsystems Pty Ltd
Unit 3, 112-118 Talavera Road
NORTH RYDE NSW 2113

Nufarm



Nufarm Australia Limited and the link with BASF Australia Limited.

Nufarm has become a successful crop protection company based in Australia but now with global activities that place it at number eight in the global ranking of agrochemical companies. The Nufarm head office is based at Laverton North in Victoria.

In 2004 Nufarm entered into an agreement with BASF Australia Limited for Nufarm to market and develop BASF products within Australia. BASF has an excellent record for developing new horticultural products, especially the discovery of new fungicides.

For further information on the Nufarm/BASF range of products contact:
doug.wilson@au.nufarm.com

FOR MORE INFORMATION:

Doug Wilson
R&D Projects Co-ordinator
Nufarm Australia Limited

Telephone (03) 9282 1427
Facsimile (03) 9282 1022
Mobile 0427 806 386
e-mail: doug.wilson@au.nufarm.com

Conference information

Registration desk

The registration desk is located in the Concert Hall Foyer of City Hall. Please direct any questions you may have regarding registration, attendance, accommodation or social functions to the staff at this desk. The registration desk will be open during the following hours:

Monday 28 September	1730–1900 (Newcastle Art Gallery)
Tuesday 29 September	0800–1900
Wednesday 30 September	0800–1730
Thursday 1 October	0800–1730

The registration desk can be contacted during these hours on 0448 576 105.

Name badges

Your name badge is your entry to all sessions, exhibition, lunches and morning and afternoon teas. *Please wear it at all times.*

Catering

Morning and afternoon teas and lunches will be held in the Banquet Room, which is located on the ground floor of City Hall. Lunches will be served as an informal stand-up buffet. We have arranged for special meals to be prepared for those delegates who have pre-registered their special requirements. These meals will be available from the designated buffet stations during meal breaks. Please see a member of the banquet staff for assistance.

Program changes

The conference organisers cannot be held responsible for any program changes due to external or unforeseen circumstances. Please check the program board located outside the Concert Hall for any changes to sessions.

Speakers preparation area

A speaker preparation area is located in the Concert Hall Foyer of City Hall and will be open during the following hours:

Monday 28 September	1730–1900 (Newcastle Art Gallery)
Tuesday 29 September	0800–1900
Wednesday 30 September	0800–1730
Thursday 1 October	0800–1600

All speakers must take their presentation to the speaker preparation area a minimum of four hours prior to their presentation, or the day before if presenting at a morning session. Speakers are also requested to assemble in their session room 15 minutes before the commencement of the session, to meet with their session chair and to familiarise themselves with the room and the audiovisual equipment.

Noticeboard

A noticeboard will be maintained adjacent to the registration desk showing program changes, messages and other information. Please check the board regularly for updates.

Mobile phones

As a courtesy to speakers and other delegates, please ensure that all mobile phones are switched off during sessions.

Participant list

The participant list has been included in the conference satchel. Those delegates who have indicated on their registration form that they do not wish to have their name and organisation appear on the participant list have not been included.

General information

Useful telephone numbers

TAXIS

Newcastle Taxis 13 33 00

HOTELS

Crowne Plaza Newcastle 4907 5065
Travelodge Newcastle 4926 3777
Ibis Newcastle 4925 2266

PUBLIC TRANSPORT

Buses 13 15 00
www.newcastlebuses.info/timetables.htm

AIRLINES

Qantas 13 13 13
Virgin Blue 13 67 89
Jetstar 13 15 38
Brindabella Airlines 1300 66 88 24

Eating out in Newcastle

Newcastle has a great food scene, with eateries to suit all budgets. There are four main dining precincts to explore in the inner city:

- Darby Street in Cooks Hill (5–10 min walk from City Hall). A diverse, friendly, relaxed bohemian precinct. Darby Street has a vibrant cafe culture, and a good selection of restaurants, pubs and take away outlets.
- Honeysuckle and the Harbour waterfront (5–10 min walk from City Hall). Down at the waterfront you will find cafes, bars and restaurants, with wonderful views across the wharves. The foreshore promenade offers a great way to walk off dessert!
- Beaumont Street in Hamilton (10 min drive from City Hall). There is a strong Mediterranean focus along Beaumont Street, with many sidewalk cafes and a thriving pub-scene.
- The Junction (25 min walk / 5 min drive from City Hall). An upmarket shopping precinct with a smattering of first-class restaurants and cafes to relax in.

Dining options closest to City Hall are:

- Civic Precinct, which has a few coffee shops and sandwich bars
- Honeysuckle and Derby Street, which both have a great selection of sit-down cafes, bars and restaurants.

For further information on places to eat in Newcastle please visit www.eatlocal.com.au/.

Things to do in Newcastle

CAROLE FRAZERS WALKS AND TALKS

Discover what makes Newcastle unique and discover Newcastle's best hidden treasures with Carole Frazer's Walks and Talks. You may be surprised that Newcastle has many fascinating walks in and around Newcastle city. Included are the spectacular Bogey Hole, Leadlight Tower and historic houses, Art Gallery and cultural buildings. All walks include commentary on many city topics and especially of local Newcastle history. There are a number of different types of walks you can do catering for a diverse range of areas and interesting locations. Prices start at \$10 per person for a 1 hour tour. For more information, log onto www.walks-talks.com.au or phone Carole on 02 4952 1537

BLACKBUTT RESERVE

Blackbutt Reserve provides nature trails, wildlife exhibits, children's playgrounds and recreational facilities. It is the perfect place for a relaxing family picnic or to explore the wonders of nature. Wildlife Exhibits open 9.00 am to 5.00 pm every day of the year. Picnic and recreation facilities open from 7.00 am to 5.00 pm and entry is free. For more information log onto www.ncc.nsw.gov.au/discover_newcastle/blackbutt_reserve

NEWCASTLES FAMOUS TRAM

Everything about Newcastle's Famous Tram is unique. Built from scratch in 1994, the tram is a genuine replica of the original Newcastle working tram, which was in service in 1923. Newcastle's Famous Tram is a very novel and nostalgic way to visit the historical city of Newcastle. The Newcastle tour is a 45 minute tour of our city, beaches and historical sites. A full commentary is provided. This service in the heart of Newcastle reveals to its passengers the beauty of the city and beach areas as well as an astonishing blend of history and current changes to the city lifestyle. Detailed information is provided about many historic sites. The tour is great value at \$12 an adult and \$6 and operates weekday tours from Newcastle's Railway Station and the Crown Plaza in Wharf Road. The Tram operates at 11.00 am and 1.00 pm, with a special pick up at the Brewery Wharf Road at 12.55 pm, and during school holidays between 10.00 am 11.00 am 12 noon and 1.00 pm, but please ring to confirm operating times. No service on weekends or public holidays. For more information log onto www.famous-tram.com.au

DARBY ST PRECINCT

Conveniently situated and only 5 minutes from Newcastle Harbour and Foreshore, Darby St Precinct offers a diverse, friendly, and relaxed cosmopolitan destination. Consisting of over 20 cafes, outdoor dining and cosy retreats, including some award winning restaurants that boast the fine cuisine with friendly prices. Shoppers look out for unique fashion boutiques, art and gift galleries. You also have photography studios, home-wares, everyday living, music and professional services. For more information log onto www.darbystreet.com.au

Social program

Welcome Reception

Monday 28 September 2009
5.30 pm – 7.00 pm

Venue: Level 1, Newcastle Region Art Gallery, 1 Laman Street, Newcastle (opposite City Hall)

Dress: Conference attire/neat casual

Marking the opening of the conference, drinks and canapés will be served in the Newcastle Region Art Gallery. The welcome reception will give you the opportunity to register early and catch up with friends.

Poster, Wine and Cheese Night

Tuesday 29 September 2009
6.00 pm – 7.00 pm

Venue: Banquet Room and Concert Hall, Newcastle City Hall

Dress: Conference attire/neat casual

Cost: Included in full conference registration. \$25 for extra attendees or other registration categories. If you wish to attend, please check with the registration desk staff if there are still tickets available.

Conference Dinner

Wednesday 30 September 2009
7.00 pm (for 7.30 pm start) until late

Venue: Auditorium 1, Newcastle Panthers Club, corner King and Union Streets, Newcastle (5 minutes walk from City Hall)

Theme: Ruby—celebrating the 40th Anniversary of the APPS

Dress: Smart casual (wear something ruby)

Cost: Included in full conference registration. \$110 for extra attendees or other registration categories. If you have not indicated on your registration form that you would like to attend, please see the registration desk staff to find out if there are still places or tickets available for purchase.

It is not every day we turn 40. Come and help us celebrate our Ruby Anniversary at the Newcastle Panthers Club. You are assured of a night of great food, great wines, fun dancing and excellent company. Let's paint Newcastle Ruby.

Beach Party

Thursday 1 October 2009
6.30 pm – 10.30 pm

Venue: Newcastle Surf Life Saving Club

Dress: Casual

Cost: The cost of the Beach Party is not included in registration fees. Cost to all delegates and guests is \$55.. If you would like to attend, please check with the registration desk staff if there are still tickets available for purchase.

Coach transfer: Departs from the front of City Hall, King Street at 5.30 pm sharp and will return at 10.30 pm. Please be waiting at the front of City Hall at least 5 minutes before the scheduled departure time.



APPS ACPP DARWIN 2011

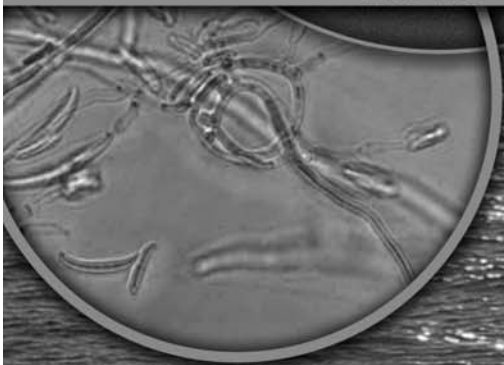
New Frontiers in Plant Pathology
for Asia and Oceania

27-29 April 2011

Darwin Convention Centre

DARWIN, NT

18th BIENNIAL AUSTRALASIAN PLANT PATHOLOGY CONFERENCE
AND THE 4th ASIAN CONFERENCE ON PLANT PATHOLOGY



The McAlpine lecture

The invitation to present the McAlpine lecture to the biennial conference of the Australasian Plant Pathology Society is extended to an eminent scientist in recognition of their significant contribution to Australasian plant pathology. The lecture is named after Daniel McAlpine, considered to be the father of plant pathology in the Australasian region. His most notable contributions were to study wheat rust following the 1889 epidemic, to classify and describe Australian smuts, and to recognise *Ophiobolus graminis* (now *Gaeumannomyces graminis*) as the cause of wheat take-all. He also collaborated with Farrer on resistance to rust in wheat (John Randles 1994, Stanislaus Fish 1976). Daniel McAlpine also contributed extensively to vegetable pathology. It is therefore fitting that a plant pathologist with extensive experience and passion such as Phil Keane be asked to deliver the McAlpine lecture in 2009.

- 1976 Dr Lilian Fraser, Department of Agriculture, NSW
Disease of citrus trees in Australia—the first hundred years
- 1978 Dr David Griffin, Australian National University, ACT
Looking ahead
- 1980 Mr John Walker, Department of Agriculture, NSW
Taxonomy, specimens and plant disease
- 1982 Professor Richard Matthews, The University of Auckland, NZ
Relationships between plant pathology and molecular biology
- 1984 Professor Bob McIntosh, University of Sydney, NSW, and Dr Colin Wellings, Department of Agriculture, NSW
Wheat rust resistance: the continuing challenge
- 1986 Dr Allen Kerr, Waite Agricultural Research Institute, SA
Agrobacterium: pathogen, genetic engineer and biological control agent
- 1989 Dr Albert Rovira, CSIRO Division of Soils, SA
Ecology, epidemiology and control of take-all, rhizotomies bare patch and cereal cyst nematode in wheat
- 1991 Mr John Walker, Department of Agriculture, NSW
Plants, diseases and pathologists in Australasia—a personal view
- 1993 Dr John Randles (University of Adelaide, SA)
Plant viruses, viroids and virologists of Australasia
- 1995 Dr Ron Close, Lincoln University, NZ
The ever changing challenges of plant pathology
- 1997 Professor John Irwin, CRC Tropical Plant Pathology, Qld
Biology and management of Phytophthora spp. attacking field crops in Australia
- 1999 Dr Dorothy Shaw, Department of Primary Industries, Qld
Bees and fungi with special reference to certain plant pathogens
- 2001 Dr Alan Dube, South Australian Research and Development Institute, SA
Long-term careers in plant pathology
- 2003 Dr Mike Wingfield, University of Pretoria, South Africa
Increasing threat of disease to exotic plantation forests in the southern hemisphere
- 2005 Dr Gretna Weste, University of Melbourne, Vic
A long and varied fungal foray

- 2007 Dr Graham Stirling, Biological Crop Protection, Qld
The impact of farming systems on soil biology and soil-borne diseases: examples from the Australian sugar and vegetable industries, the case for better integration of sugarcane and vegetable production and implications for future research
- 2009 Assoc Prof Phil Keane, La Trobe University, Vic
Lessons from the tropics—the unfolding mystery of vascular-streak dieback of cocoa, the importance of genetic diversity, horizontal resistance, and the plight of farmers

McAlpine lecturer 2009: Philip Keane



Philip grew up in the wheat/sheep belt of rural South Australia and gained his Bachelor of Agricultural Science (Hons) at the Waite Agricultural Research Institute, University of Adelaide in 1968.

He was awarded a PhD at the University of Papua New Guinea in 1972 for his studies of vascular-streak dieback, a serious epidemic disease of cocoa. He described and named the pathogen, *Oncobasidium theobromae*,

and remains the world authority on what is a particularly unusual vascular wilt disease. Not only was the pathogen a new species, but also a new genus within the Basidiomycetes.

Philip taught at UPNG before taking up a lectureship at La Trobe University in 1975. His time at La Trobe has been supplemented with sabbatical periods in the USA and Central America, as well as extensive project-related travel through PNG and Indonesia.

Since returning to Australia in 1975 Philip maintained his interest in diseases of cocoa in South East Asia and Papua New Guinea, and in agricultural development and education in tropical countries. His approach is focussed on the farmer—from listening to farmers, evaluating their ideas, then translating his research to be used by the farmers. Philip also initiated research into fungal diseases of crop plants and eucalypts, and co-edited the standard monograph on Eucalypt Pathogens and Diseases. He is involved in research on a range of big questions in plant pathology, including the nature of resistance to crop diseases, especially cereal rusts, plant disease epidemiology, the diversity of macrofungi and broad questions in plant ecology.

Philip is an enthusiastic undergraduate teacher and has trained many local and international PhD students, many of whom will be attending this conference. He has made a special and unique contribution to plant pathology in Australia and neighbouring countries, and it is a great honour that he has accepted our invitation to present the McAlpine Lecture.

Keynote biographies

Barbara Christ

Professor Barbara Christ, the current President of the American Phytopathological Society, is Senior Associate Dean in the College of Agricultural Sciences and Professor of Plant Pathology at Pennsylvania State University in the United States. Her research is focused on potato breeding and disease management, including basic research into understanding the inheritance of disease resistance as well as extension. Her research includes developing and releasing new varieties adapted for Pennsylvania growing conditions, developing disease-resistant potato germplasm, examining the genetic variability and biology of potato pathogen populations, developing methods to detect and forecast potato diseases, developing integrated pest management strategies for potatoes in Pennsylvania, and evaluating new fungicides for efficacy against potato diseases.

André Drenth

Dr André Drenth is a Principal Plant Pathologist from the University of Queensland, and founder and Leader of the Tree Pathology Centre which is a joint initiative between the University of Queensland and Queensland Primary Industries and Fisheries. André studied Plant Breeding and Pathology at Wageningen University and Cornell University, USA. André was Research Program Leader in the CRC for Tropical Plant Protection dealing with a large number of Tropical diseases. His ability to deliver practical outcomes from basic research in plant pathology is well recognised internationally. André has been involved in research on plant pathogens for nearly 20 years and has published widely on a range of plant diseases with a special focus on *Phytophthora*.

Adrienne Hardham

Professor Adrienne Hardham works in the Research School of Biology at the Australian National University. The main focus of her research is on cellular and molecular mechanisms responsible for the infection of plants by *Phytophthora* and rust fungi and the plant's defence response to pathogen invasion.

Greg Johnson

Dr Greg Johnson is President of the Australasian Plant Pathology Society (APPS) 2007–2009 and Secretary General of the International Society for Plant Pathology (ISPP) 2006–2013. Greg has had over 20 years' experience in development assistance in tropical horticulture and postharvest R&D collaboration with developing countries in Asia and the Pacific, and over 30 years' experience in plant pathology practice, diagnostic advice and publishing on tropical and temperate plants and crops. His especial interest is postharvest diseases of mangoes. Greg currently operates a Canberra-based consultancy, Horticulture 4 Development, that builds upon Greg's background in managing a portfolio of projects and activities in postharvest technology, horticulture and crop protection with the Australian Centre for International Agricultural Research (ACIAR) in Asia and the Pacific. His recent activities have included an overview of the vegetable sector in tropical Asia and reviewing issues and priorities for postharvest disease management in mangoes.

Celeste Linde

Celeste Linde investigates the population genetics of, for example, cereal pathogens, the influence of wild or weedy hosts on pathogen populations and their evolution of virulence. Her main focus has been with *Rhynchosporium secalis*, causing barley scald.

Eun Woo Park

Professor Eun Woo Park is Dean of the College of Agriculture and Life Sciences in Seoul National University, Korea. Major research areas are epidemiology of airborne diseases with special emphasis on modeling and forecasting disease development, and applications of various information technologies to implement disease management strategies.

Dov Prusky

Professor Dov Prusky is Deputy Director Research and Development with the Agricultural Research Organization, Israel. He is also active in research in the Department of Postharvest Science of Fresh Produce of the ARO Technology and Storage of Agricultural Products Institute. Dov is currently Chair of the ISPP Postharvest Diseases Subject Matter committee. Dov's research interests include:

- understanding the basic processes underlying the interactions between fruits and pathogenic fungi
- studying biochemical and molecular mechanisms that are controlled by fungal virulence and fruit resistance factors
- using transformation-mediated gene disruption to create strains of pathogenic fungi that are specifically mutated in their ability to make cell-wall degrading enzymes and other pathogenicity factors. These mutants are tested for their ability to cause disease and to elicit defense responses
- studying the biochemical basis for modulation of pathogenicity factor affecting the transcription expression of nitrogen metabolism, ammonia secretion and the effect on the modulation of local pH
- reduction of postharvest losses in deciduous and subtropical fruits.

Robert Seem

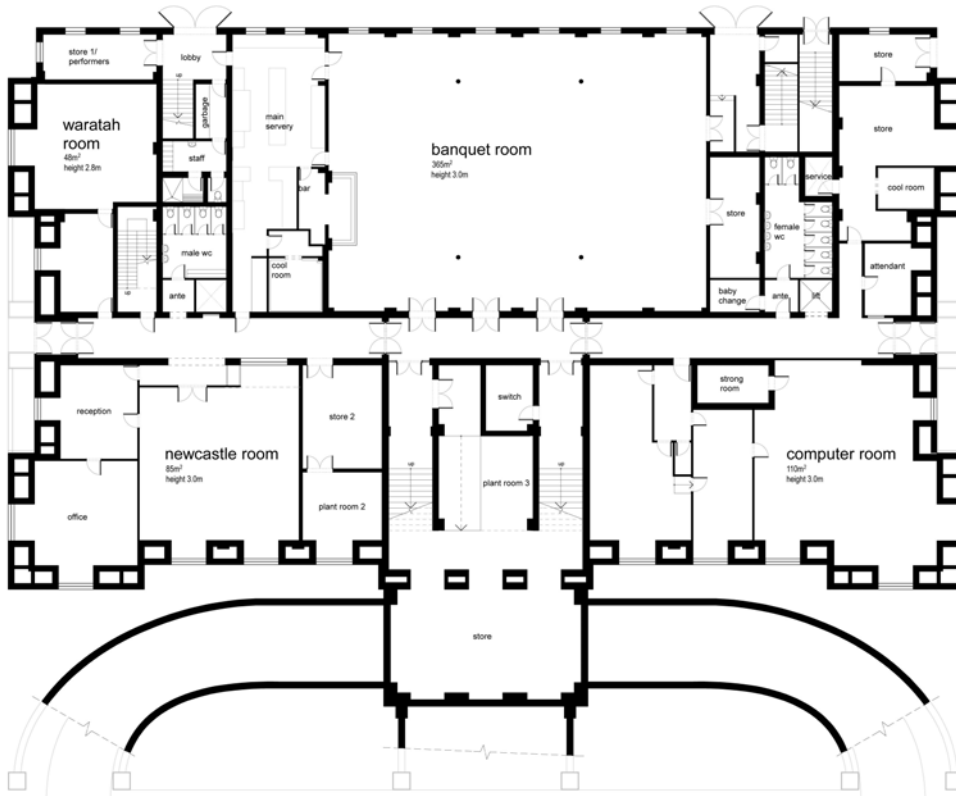
Robert C Seem has spent his 34-year career as professor of plant pathology at Cornell University's Agricultural Experiment Station in Geneva, New York. He specialises in the epidemiology of fruit and vegetable diseases. Robert also served in the station administration for 14 years. During this time he was instrumental in the development of the Cornell Agriculture and Food Technology Park Corporation, where he continues to serve a president of the board.

Mike Wingfield

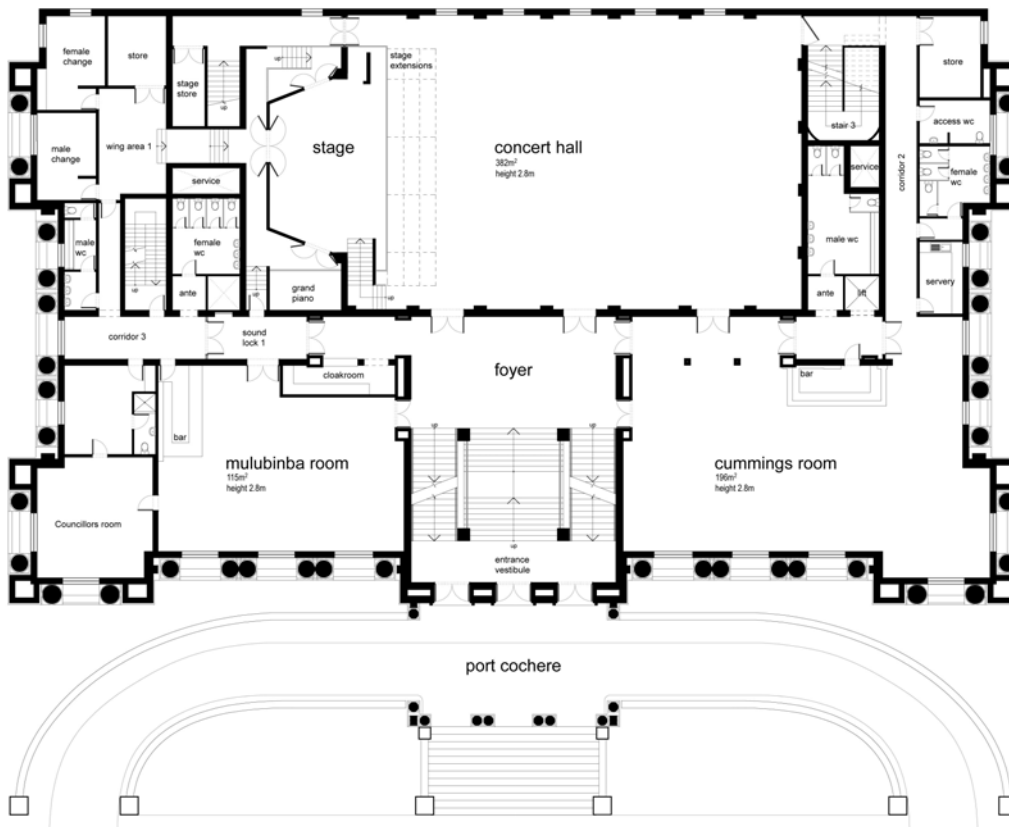
Professor Michael Wingfield was born in South Africa. He graduated with BSc Hons (Natal) and MSc (Stellenbosch) degrees then completed his PhD in Plant Pathology (University of Minnesota), specialising in forest pathology and forest entomology. He returned to South Africa to establish the Tree Protection Co-operative Programme (TPCP) at the University of the Free State, and in 1998 established the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria. FABI is now the Centre of Excellence in Tree Health Biotechnology. He is also an alumnus of the Harvard Business School Advanced Management Programme.

Venue map

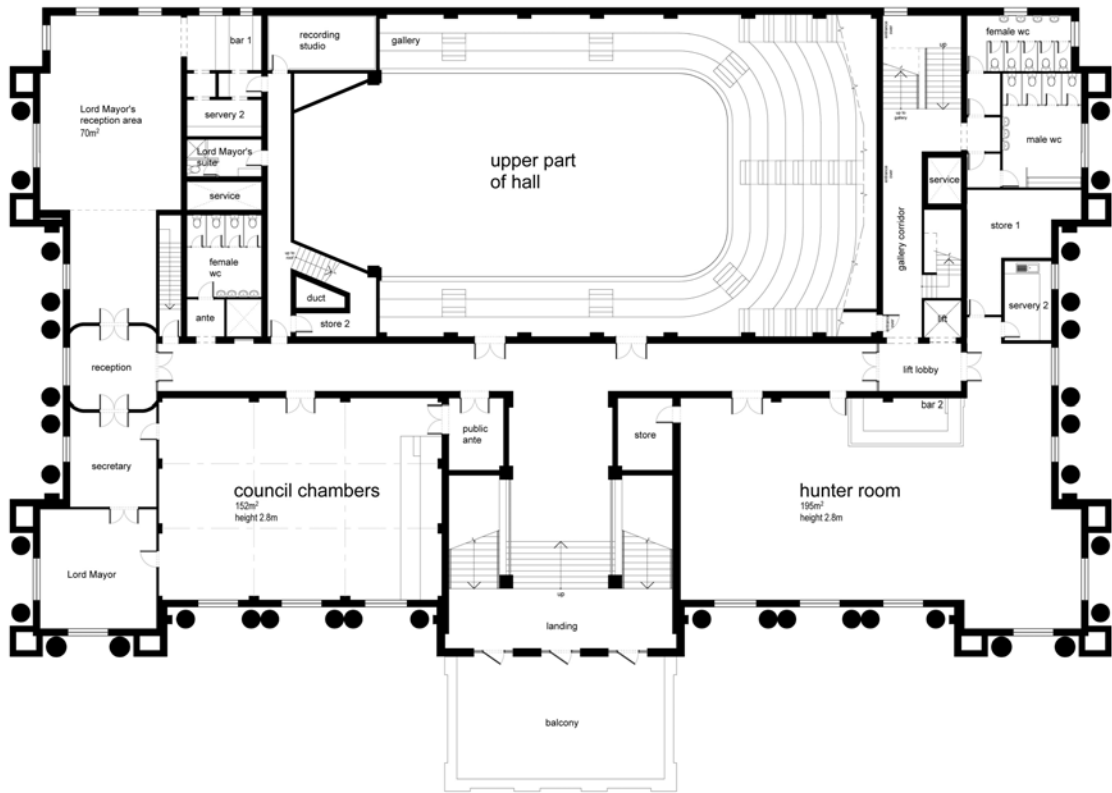
Level 1



Level 2



Level 3



Program

Monday 28 September

1400–1700	Registration open	Ground Floor Foyer, Newcastle Region Art Gallery
1730–1800	WELCOME RECEPTION	Newcastle Region Art Gallery



Tuesday 29 September


0800–1900	Registration open	Concert Hall Foyer																
0800	ARRIVAL TEA AND COFFEE	Concert Hall Foyer																
0830	Conference opening Prof David Guest , University of Sydney	Concert Hall																
0845	Presidential address— <i>‘Shield the young harvest from devouring blight’</i> —Charles Darwin, Joseph Banks, Thomas Knight and wheat rust: discovery, adventure, and ‘getting the message out’ Dr Greg Johnson , Horticulture 4 Development, ACT	Concert Hall																
0930	Keynote address— <i>The relevance of plant pathology in food production</i> Dr André Drenth , Tree Pathology Centre, The University of Queensland and Primary Industries and Fisheries	Concert Hall																
1030	MORNING TEA	Banquet Room																
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Session 1A Disease management Room: Concert Hall Chair: Andrew Miles	Session 1B Disease surveys Room: Cummings Room Chair: Eileen Scott	Session 1C Soilborne diseases Room: Hunter Room Chair: Nerida Donovan	Session 1D Virology Room: Newcastle Room Chair: John Randles															
1100 <i>An integrated approach to husk spot management in macadamia</i> Dr Olufemi Akinsanmi , The University of Queensland and Primary Industries and Fisheries, Qld	<i>Why Australia needs a coordinated national diagnostic system</i> Ms Jane Moran , Department of Primary Industries, Vic	<i>Can investment in building up soil organic carbon lead to disease suppression in vegetable crops?</i> Dr Ian Porter , Department of Primary Industries, Vic	<i>Towards universal detection of Luteoviridae</i> Miss Anastasija Chomic , Lincoln University, NZ															
1120 <i>Application methods of phosphonate to control Phytophthora pod rot and stem canker on cocoa</i> Dr Peter McMahan , La Trobe University, Vic	<i>Development of a soil DNA extraction and quantitative PCR method for detecting two Cylindrocarpon species in soil</i> Ms Chantal Probst , Lincoln University, NZ	<i>Evaluation of soil health indicators in the vegetable industry of temperate Australia</i> Ms Robyn Brett , Department of Primary Industries, Vic	<i>Massive parallel sequencing of small RNAs to identify plant viruses and virus-induced small RNAs</i> Dr Robin MacDiarmid , The New Zealand Institute for Plant and Food Research Ltd, NZ															
1140 <i>Botrytis bunch rot control strategies in cool climate viticultural regions of Australia and New Zealand</i> Dr Jacqueline Edwards , Department of Primary Industries, Vic	<i>Bananas in Carnarvon—good news for growers in survey for quarantine plant pests and pathogens</i> Dr Sarah Collins , Department of Agriculture and Food WA	<i>Rhizoctonia AG2.1 and AG3 in soil—competition or synergism?</i> Dr Tonya Wiechel , Department of Primary Industries, Vic	<i>Chickpea chlorotic stunt virus, an important virus of cool-season food legumes in Asia and North Africa and potentially in Australia</i> Dr Safaa Kumari , International Center for Agricultural Research in the Dry Areas, Syria															
1200	LUNCH Editor’s meeting (1200–1400) Student mentor lunch (1200–1320)	Banquet Room Waratah Room Mulumbinba Room																
1320	Keynote address— <i>Emerging frontiers in forest pathology</i> Prof Mike Wingfield , Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa	Concert Hall																



	Session 2A Forest pathology/native Room: Concert Hall Chair: Angus Carnegie	Session 2B Soilborne disease Room: Cummings Room Chair: Peter McGee	Session 2C Epidemiology Room: Hunter Room Chair: Chris Steel	Session 2D Disease management Room: Newcastle Room Chair: Robert Magarey
1410	<i>Variability in pathogenicity of Quambalaria pitereka on spotted gums</i> Mr Geoffrey Pegg , The University of Queensland/ Primary Industries and Fisheries, Qld	<i>Optimising conditions to investigate gene expression in pathogenic Streptomyces using RT-qPCR</i> Dr Tonya Wiechel , Department of Primary Industries, Vic	<i>Bunch rot diseases and their management</i> Prof Turner Sutton , NC State University, USA	<i>Sugarcane smut—disease development and mechanism of resistance</i> Dr Shamsul Bhuiyan , BSES Limited, Qld
1430	<i>Movement of pathogens between horticultural crops and endemic trees in the Kimberleys</i> Ms Monique Sakalidis , Murdoch University, WA	<i>Fusarium oxysporum and Pythium associated with vascular wilt and root rots of greenhouse cucumbers</i> Mr Len Tesoriero , NSW Department of Primary Industries	<i>Inoculum and climatic factors driving epidemics of Botrytis cinerea in New Zealand and Australian vineyards</i> Dr Rob Beresford , The New Zealand Institute for Plant and Food Research Limited, NZ	<i>Dissemination of biological and chemical fungicides by bees onto Rubus and Ribes flowers</i> Dr Monika Walter , The New Zealand Institute for Plant and Food Research Limited, NZ
1450	<i>Pathogenicity of Phytophthora multivora to Eucalyptus gomphocephala and E. marginata</i> Dr Treena Burgess , Murdoch University, WA	<i>Fusarium oxysporum f. sp. fragariae: a main component of strawberry crown and root rots in Western Australia</i> Dr Hossein Golzar , Department of Agriculture and Food WA	<i>Infection of apples by Colletotrichum acutatum in New Zealand is limited by temperature</i> Dr Kerry Everett , The New Zealand Institute for Plant and Food Research Limited, NZ	<i>Current studies on divergence and management of pepper yellow leaf curl disease Indonesia</i> Dr Sri Hidayat , Bogor Agricultural University, Indonesia
1510	<i>Microscopy of progressive decay of fungi isolated from Meranti tree canker</i> Dr Erwin Erwin , University of Mulawarman, Indonesia	<i>Evaluation of resistant rootstocks for control of Fusarium wilt of watermelon in Nghe An Province, Vietnam.</i> Prof Lester Burgess , The University of Sydney, NSW	<i>Epidemiology of walnut blight, caused by Xanthomonas arboricola pv. juglandis, in Tasmania, Australia</i> Dr Katherine Evans , University of Tasmania	<i>Fungicide resistance in cucurbit powdery mildew</i> Dr Chrys Akem , Primary Industries and Fisheries, Qld
1530	AFTERNOON TEA			Banquet Room
1600	Keynote address— <i>Population genetic analyses of plant pathogens: new challenges and opportunities</i> Dr Celeste Linde , Research School of Biology, College of Medicine, Biology and Environment, Australian National University			Concert Hall
	Session 3A Population genetics Room: Concert Hall Chair: Andre Drenth	Session 3B Modelling and crop loss assessment Room: Cummings Room Chair: Ian Porter	Session 3C Disease management Room: Hunter Room Chair: Shane Hetherington	
1640	<i>Genetic diversity of Botryosphaeria parva (Neofusicoccum parvum) in New Zealand vineyards</i> Mr Jeyaseelan Baskarathevan , Lincoln University, NZ	<i>Spore traps for early warning of smut infestations in Australian sugarcane crops</i> Dr Rob Magarey , BSES Limited, Qld	<i>Management of white blister on vegetable brassicas with irrigation and varieties</i> Dr Elizabeth Minchinton , Department of Primary Industries, Vic	
1700	<i>Anthraxnose disease of chili pepper—genetic diversity, pathogenicity and breeding for resistance</i> A/Prof Paul Taylor , The University of Melbourne, Vic	<i>Software-assisted gap estimation (SAGE) for measuring grapevine leaf canopy density</i> Mr Gareth Hill , The New Zealand Institute for Plant and Food Research Limited, NZ	<i>Alternative screening methods for sugarcane smut using natural infection and tissue staining</i> Dr Shamsul Bhuiyan , BSES Limited, Qld	
1720	<i>The diversity of Colletotrichum infecting lychee in Australia</i> Ms Jay Anderson , Primary Industries and Fisheries, Qld and University of Queensland	<i>Evaluation of the efficacy of Brassica_{spot}TM models for control of white blister in Chinese cabbage</i> Mr Desmond Auer , Department of Primary Industries, Vic	<i>Interruption of cool chain and strawberry fruit rot by leak-causing fungi Rhizopus species</i> Dr Monika Walter , The New Zealand Institute for Plant and Food Research Limited, NZ	
1740	<i>Variation in Phytophthora palmivora on cocoa in Papua New Guinea</i> Ms Josephine Saul Maora , PNG Cocoa Coconut Institute	<i>Evaluating an infection model of prune rust to improve the management of disease for almond and prune growers</i> Mr Peter Magarey , South Australian Research and Development Institute	<i>Enhancing Papua New Guinea smallholder cocoa production through greater adoption of integrated pest and disease management</i> Mr Yak Namaliu , PNG Cocoa Coconut Institute	
1800	DRINKS AND POSTERS			Banquet Room and Concert Hall
1830–2030	Council of Society meeting			Waratah Room

Wednesday 30 September

0800–1730	Registration open	Concert Hall Foyer	
0800	ARRIVAL TEA AND COFFEE	Concert Hall Foyer	
0830	Keynote address— <i>Molecular cytology of Phytophthora-plant interactions</i> Prof Adrienne Hardham , Plant Cell Biology Group, Research School of Biology, The Australian National University	Concert Hall	
	Session 4A Plant pathogen interactions Room: Concert Hall Chair: David Guest	Session 4B Disease surveys Room: Cummings Room Chair: Sandra Savocchia	Session 4C Epidemiology Room: Hunter Room Chair: Greg Johnson
	Session 4D Prokaryotic pathogens Room: Newcastle Room Chair: Lucy Tran-Nguyen		
0910	<i>Gene expression changes during host-pathogen interaction between Arabidopsis thaliana and Plasmodiophora brassicae</i> Mrs Arati Agarwal , Department of Primary Industries, Vic	<i>Prevalence and pathogenicity of Botryosphaeria lutea isolated from grapevine nursery materials in New Zealand</i> Ms Regina Billones , Lincoln University, NZ	<i>Honey bees— do they aid the dispersal of Alternaria radicina in carrot seed crops?</i> Mr Rajan Trivedi , Lincoln University, NZ
			<i>Transmission of 'Candidatus Phytoplasma australiense' to Cordyline and Coprosma</i> Dr Ross Beever , Landcare Research, NZ
0930	<i>Hairpin RNA derived from viral Nla gene confers immunity to wheat streak mosaic virus infection in transgenic wheat plants</i> Mr Muhammad Fahim , CSIRO Plant Industry, and Australian National University, ACT	<i>Infection and disease progression of Neofusicoccum luteum in grapevine plants</i> Mr Nicholas Amponsah , Lincoln University, NZ	<i>Translating research into the field: meta-analysis of field pea blackspot severity and yield loss to extend model application for disease management in Western Australia</i> Dr Moin Salam , Department of Agriculture and Food WA
			<i>Australian grapevine yellows phytoplasma found in symptomless shoot tips after a heat wave in South Australia</i> Mr Peter Magarey , South Australian Research and Development Institute, SA
0950	<i>Characterising inositol signalling pathways in Phytophthora spp. for future development of selective antibiotics</i> Mr Dean Phillips , Deakin University, Vic	<i>Carbohydrate stress increases susceptibility of grapevines to Cylandrocarpon black foot disease</i> Miss Dalin Dore , Lincoln University, NZ	<i>Development of a model to predict spread of exotic wind and rain borne fungal pests</i> Dr Moin Salam , Department of Agriculture and Food WA
			<i>Association of Phytoplasmas with papaya crown yellows (PCY) disease—a new disease of papaya in Northern Mindanao, Philippines</i> Ms Regina Billones , Del Monte Phils Inc, Philippines
1010	<i>Systemic acquired resistance—a new addition to the IPM clubroot toolbox?</i> Dr Caroline Donald , Department of Primary Industries, Vic	<i>Botryosphaeria spp. associated with bunch rot of grapevines in south-eastern Australia</i> Ms Nicola Wunderlich , Charles Sturt University, NSW	<i>Psyllid transmission of Huanglongbing from naturally infected Shogun mandarin to orange jasmine</i> Dr Rantana Sdoodee , Prince of Songkla University, Thailand
			<i>Phytoplasma diseases in citrus orchards of Pakistan</i> Dr Shazia Mannan , COMSATS Institute of Information Technology, Pakistan
1030	MORNING TEA	Banquet Room	
1100	Keynote address— <i>Mechanisms modulating fungal attack in postharvest pathogen interactions and their modulation for improved disease control</i> Prof Dov Prusky , Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, Israel	Concert Hall	
	Session 5A Plant pathogen interactions Room: Concert Hall Chair: Rosalie Daniel	Session 5B Disease surveys Room: Cummings Room Chair: Aaron Maxwell	Session 5C Chemical control Room: Hunter Room Chair: Len Tesoriero
1140	<i>ABA-dependant signalling of PR genes and potential involvement in the defence of lentil to Ascochyta lentis</i> Dr Rebecca Ford , The University of Melbourne, Vic	<i>Fishing For Phytophthora across Western Australia's water bodies</i> Dr Daniel Hüberli , Murdoch University, WA	<i>Evaluation of fungicides to manage brassica stem canker</i> Ms Lynette Deland , South Australian Research and Development Institute, SA
1200	<i>Fundamental components of resistance to Phytophthora cinnamomi: using model system approaches</i> Prof David Cahill , Deakin University, Vic	<i>Incidence of fungi isolated from grape trunks in New Zealand vineyards</i> Mr Dion Mundy , The New Zealand Institute for Plant and Food Research Limited, NZ	<i>Evaluation of spray programs for powdery mildew management in greenhouse cucumbers</i> Dr Kaye Ferguson , South Australian Research and Development Institute, SA
1220	<i>Genes involved in hypersensitive cell death responses during Fusarium crown rot infection in wheat</i> Dr Jill Petrisko , University of Southern Queensland, Qld	<i>Isolation and characterisation of strains of Pseudomonas syringae from waterways of the Central North Island of New Zealand</i> Dr Joel Vanneste , The New Zealand Institute for Plant and Food Research Limited, NZ	<i>The incidence of copper resistant bacteria in Australian pome and stone fruit orchards</i> Dr Chin Gouk , Department of Primary Industries, Vic

1240	AGRICHEM LUNCH		Banquet Room
1340	Poster session		Banquet Room and Concert Hall
1430	AFTERNOON TEA		Banquet Room
1500	McAlpine lecture— <i>Lessons from the tropics—the unfolding mystery of vascular-streak dieback of cocoa, the importance of genetic diversity, horizontal resistance, and the plight of farmers</i> Assoc Prof Phil Keane , Department of Botany, La Trobe University, Vic		Concert Hall
1600	AGM		Concert Hall
1730	Close of day		
1900	CONFERENCE DINNER		Newcastle Panthers Club

Thursday 1 October

0700	Regional Councillor's meeting	Waratah Room	
0700	CHAIRMAN'S BREAKFAST	Mulumbinba Room	
0800–1730	Registration open	Concert Hall Foyer	
0800	ARRIVAL TEA AND COFFEE	Concert Hall Foyer	
0830	Keynote address— <i>Translating research into the field: how it started, how it is practised and how we carry out grape powdery mildew research</i> Dr Bob Seem , Cornell University, USA	Concert Hall	
0910	GRDC book launch: Mr James Clarke , Grains Research and Development Corporation	Concert Hall	
	Session 6A Cereal pathology 1 Room: Concert Hall Chair: Mark Sutherland	Session 6B Quarantine and exotic pathogens Room: Cummings Room Chair: Suzy Perry	Session 6C Alternatives to chemical control Room: Hunter Room Chair: Carolyn Blomley
0925	<i>Stem rust race Ug99: international perspectives and implications for Australia</i> Dr Colin Wellings , The University of Sydney, NSW	<i>Development of an eradication strategy for exotic grapevine pathogens</i> Dr Mark Sosnowski , South Australian Research and Development Institute, SA	<i>The influence of soil biotic factors on the ecology of Trichoderma biological control agents</i> Prof Alison Stewart , Lincoln University, NZ
0945	<i>Mitigating crop losses due to stripe rust in Australia: integrating pathogen population dynamics with research and extension programs</i> Dr Colin Wellings , The University of Sydney, NSW	<i>Green grassy shoot disease of sugarcane, a major disease in Nghe An Province, Vietnam</i> Dr Rob Magarey , BSES Limited, Qld	<i>Understanding Trichoderma bio-inoculants in the root system of Pinus radiata</i> Mr Pierre Hohmann , Lincoln University, NZ
1005	<i>Impact of sowing date on crown rot losses</i> Dr Steven Simpfendorfer , Department of Primary Industries, NSW	<i>Molecular detection of Mycosphaerella fijiensis in the leaf trash of 'Cavendish' banana</i> Dr Seona Casonato , The New Zealand Institute for Plant and Food Research Limited, NZ	<i>A bioassay to screen Trichoderma isolates for their ability to promote root growth in willow</i> Mr Mark Braithwaite , Lincoln University, NZ
1025	<i>Symptom development and pathogen spread in wheat genotypes with varying levels of crown rot resistance</i> Dr Cassandra Malligan , Queensland Primary Industries and Fisheries	<i>Optimising responses to incursions of exotic plant pathogens</i> Dr Mike Hodda , CSIRO Entomology, ACT	<i>Biofumigation for reducing Cylandrocarpon spp. in New Zealand vineyard and nursery soil</i> Ms Carolyn Bleach , Lincoln University, NZ
1045	MORNING TEA	Banquet Room	
	Session 7A Cereal pathology 2 Room: Concert Hall Chair: Colin Wellings	Session 7B Quarantine and exotic pathogens Room: Cummings Room Chair: Nerida Donovan	Session 7C Alternatives to chemical control Room: Hunter Room Chair: Alison Stewart
1100	<i>Crown rot of winter cereals: integrating molecular studies and germplasm improvement</i> Prof Mark Sutherland , University of Southern Queensland, Qld	<i>Twenty years of quarantine plant disease surveillance on the island of New Guinea: key discoveries for Australia and PNG</i> Mr Richard Davis , Australian Quarantine and Inspection Service, Qld	<i>Fruit extracts of Azadirachta indica induces systemic acquired resistance in tomato against Pseudomonas syringae pv tomato</i> Dr Prabir Paul , Amity University, India
1120	<i>Infection of wheat tissues by Fusarium pseudograminearum</i> Mr Noel Knight , University of Southern Queensland, Qld	<i>The importance of reporting suspect exotic or emergency plant pests to your State</i> Department of Primary Industry Dr Sophie Peterson , Plant Health Australia, ACT	<i>Fungal foliar endophytes induce systemic protection in cacao seedlings against Phytophthora palmivora</i> Ms Carolyn Blomley , The University of Sydney, NSW
1140	<i>Monitoring sensitivity to Strobilurin fungicides in Blumeria graminis on wheat and barley in Canterbury, New Zealand</i> Dr Suvi Viljanen-Rollinson , The New Zealand Institute for Plant and Food Research Limited, NZ	<i>The use of sentinel plantings in forest biosecurity; results from mixed eucalypt species trails in South-East Asia and Australia</i> Dr Treena Burgess , Murdoch University, WA	<i>Effectiveness of the rust Puccinia myrsiphylli in reducing populations of the invasive plant bridal creeper in Australia</i> Dr Louise Morin , CSIRO Entomology, ACT
1200	<i>Cross inoculation of crown rot and Fusarium head blight isolates of wheat</i> Mr Philip Davies , University of Sydney, NSW	<i>Methyl bromide alternatives for quarantine and pre-shipment and other purposes—future perspectives</i> Ms Janice Oliver , Office of the Chief Plant Protection Officer, ACT	<i>Evaluation of essential oils and other plant extracts for control of soilborne pathogens of vegetable crops</i> Ms Cassie Scoble , Department of Primary Industries and La Trobe University, Vic
1230	LUNCH	Banquet Room	

1330	Keynote address— <i>Use of grid weather forecast data to predict rice blast development in Korea</i> Prof Eun Woo Park , College of Agriculture and Life Sciences, Seoul National University, Korea	Concert Hall
1410	<i>Investigating the impact of climate change on plant diseases</i> Dr Jo Luck , Department of Primary Industries, Vic	
1430	<i>Impact of climate change in relation to blackleg on oilseed rape and blackspot on field pea in Western Australia</i> Dr Moin Salam , Department of Agriculture and Food, WA	
1450	<i>Approaches to training in plant pathology capacity building projects in developing countries</i> Prof LW Burgess , University of Sydney, NSW	
1510	<i>Increasing global regulations on fumigants stimulates new era for plant protection and biosecurity</i> Dr Ian Porter , Department of Primary Industries, Vic	
1530	AFTERNOON TEA	Banquet Room
1600	Keynote address— <i>A world of possibilities</i> Dr Barbara Christ , The Pennsylvania State University, USA	Concert Hall
1630	Incoming presidential address Dr Caroline Mohammed , School of Agricultural Science, University of Tasmania	
1645	Awards	
1700	Close of day	
1730	Bus leaves Civic Centre for Beach Party	
1800	BEACH PARTY	Newcastle Surf Life Saving Club

Oral abstracts

'Shield the young harvest from devouring blight'—Charles Darwin, Joseph Banks, Thomas Knight and wheat rust: discovery, adventure, and 'getting the message out'

G.I. Johnson{ XE "Johnson, G.I." }

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1969: The year of the first moon landing (20 July 1969), the Woodstock Festival in upstate New York (15–18 August 1969), and (coinciding the last day of Woodstock) the beginning of the Australasian Plant Pathology Society (first AGM at 41st ANZAAS Meeting, Adelaide (18 August 1969) (Purss 1994)). All had a lengthy gestation and challenges along the way. All have changed the world!

In the 17th President's Address to the Australasian Plant Pathology Society, David Guest (2001), noted: 'I became a plant pathologist because the mechanisms organisms use to communicate fascinate me'. Well, I became a plant pathologist because I am gardener at heart. But I have learned along the way that communication is a critical issue—not only the communication amongst and between microorganisms and plants, but also that between plant pathologists, farmers, politicians and communities. And, communication that is timely, inspiring and, (preferably) accurate, often yields the most favourable outcomes.

In this paper, I will explore some of the early communication relating to plant disease, particularly wheat rusts. I refer to Erasmus and Charles Darwin, Joseph Banks, Thomas Knight, and some pioneering Australian researchers, and the roles of conferences, publications and newspapers, to highlight how 'getting our message out' was as important in the 19th and early 20th centuries as it is now. And, finally, I will consider how a scientific society in the 21st century still has relevance and the potential to change the world.

The relevance of plant pathology in food production

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Plants are our only true primary producers of food, fibre and fuel through the process of photosynthesis. The objective of plant science in general is to understand the principles and processes involved in plant growth and reproduction and the objective of crop science concerns the productivity of our crops.

Over a period of 35 years from 1960 to 1995 the world food production doubled while the world population more than doubled from 2.5 billion to 5.6 billion. The present world population is 6.7 billion and expected to grow to 9 billion by 2050. Agricultural production needs to increase 2.3% a year just to meet global food demand. At present we increase it by 1.5% a year. Thus the challenge for Agriculture is to double the global food production over the next three decades. In addition to meeting the challenge for food production Agriculture is also expected to provide renewable fuel. It should be clear that society needs Agriculture now more than ever before.

The objective of the discipline of plant pathology is to reduce the impact of diseases on the production of plants for food, fibre and fuel. Plant pathology is an important biological science and arose out of need during times of famine, poor food security and large scale crop losses. Despite clearly defined objectives and a proud history of achievements many plant pathologists would agree with the statement 'Plant pathology in relation to its importance to humanity continues to be a grossly underfunded discipline' (Strange and Scott, 2005, Annual Review of Phytopathology). In order to ascertain the significance of our relatively small discipline we must recognise and document past contributions, identify and understand future challenges, and be actively working on tomorrow's problems. The aim of my presentation is to address the following three questions:

- What have been the contributions and impacts of plant science and more specifically plant pathology on food production?
- What are the key challenges with regards to plant production which will enable Agriculture to feed a growing world population in the future?
- What role can our discipline of plant pathology play in feeding the world?

An integrated approach to husk spot management in macadamia

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INTRODUCTION

Husk spot, caused by *Pseudocercospora macadamiae* is a major fungal disease of macadamia in Australia (3). Husk spot occurs only in eastern Australia, costing over \$10 million in lost productivity if the disease is not adequately controlled. *P. macadamiae* infects macadamia husks on which it continually produces inoculum (4), the infection causes premature abscission of diseased fruit, thus, resulting in extensive yield losses and reduced kernel quality. Application of fungicide is currently the only effective method for controlling husk spot (1). However, several factors including the upsurge in organic farming, the need for sustainable management practices, and possible development of fungicide resistant fungal strains and lack of quantitative information on levels of disease resistance in varieties require development of integrated management strategies for controlling husk spot. Systematic studies were performed to improve husk spot control through the provision of alternative fungicide, biological control options, effective cultural practices and diagnostic characters for disease resistant cultivars.

MATERIALS AND METHODS

In order to evaluate the efficacy of different fungicides and biocontrol options against husk spot, both laboratory and field trials were established. Macadamia trees treated with different fungicide products at three field sites in south east Queensland and northern New South Wales were evaluated for husk spot incidence and severity, from onset of visual symptoms to kernel maturity (2). The area under disease progress curves of the treatments were compared against each other and with the untreated control using analysis of variance. The activities of five *Trichoderma* species against *P. macadamiae* were assessed either singly or in combination with each other and bacteria (*Bacillus subtilis* and *Pseudomonas fluorescence*) in laboratory experiments. Five characters of macadamia varieties with varying incidence of husk spot were evaluated as possible diagnostic characters for husk spot resistant varieties and discriminant analysis was performed using the identified diagnostic characters to partition 12 macadamia varieties to husk spot resistance.

RESULTS AND DISCUSSION

Results of field trials showed that pyraclostrobin conferred significantly ($P < 0.05$) better protection than trifloxystrobin and also had somewhat similar efficacy as a tank-mixture of carbendazim and copper against husk spot incidence and severity. The use of pyraclostrobin in rotation with tank mixture of carbendazim and copper would play a key role in the management of fungicide resistance in the industry. The reduction of copper usage would also provide additional benefits to the macadamia industry. Frequency or number of fungicide spray applications influenced total kernel quality (Fig. 1). *In vitro* volatile inhibition trials showed that growth rate of *P. macadamiae* was inhibited by 8% in mixed cultures of *T. viride* and *T. harzianum*, and by 5% in the mixed culture of *T. koningii* and *T. harzianum* but no mycoparasitism was observed in the hyphal interaction experiments. Our results showed that significant differences exist in the reaction of macadamia varieties to husk spot. The discriminant analysis on the disease incidence and severity, prevalence of stick-tights, number of

stomata per unit area and number of lesion per fruit classified macadamia varieties into various resistance groups.

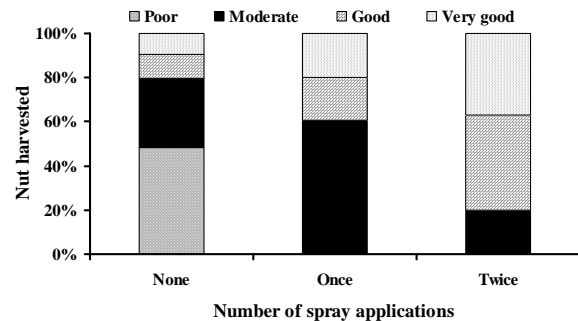


Figure 1. Proportion of quality of total macadamia kernel produced from trees that received varying number of spray applications.

ACKNOWLEDGEMENTS

We acknowledge the University of Queensland, Primary Industries and Fisheries Queensland, Australian Macadamia Society Ltd., Horticulture Australia Ltd. and Nufarm Australia Pty Ltd for the funding for this project.

REFERENCES

1. Akinsanmi, O.A., Miles, A.K., and Drenth, A. 2007. Timing of fungicide application for control of husk spot caused by *Pseudocercospora macadamiae* in macadamia. *Plant Dis.* 91:1675–1681.
2. Akinsanmi, O.A., Miles, A.K., and Drenth, A. 2008. Alternative fungicides for controlling husk spot caused by *Pseudocercospora macadamiae* in macadamia. *Australas. Plant Pathol.* 37:141–147.
3. Beilharz, V., Mayers, P.E., and Pascoe, I.G. 2003. *Pseudocercospora macadamiae* sp. nov., the cause of husk spot of macadamia. *Australas. Plant Pathol.* 32 (2):279–282.
4. Miles, A.K., Akinsanmi, O.A., Sutherland, P.W., Aitken, E.A.B., and Drenth, A. 2009. Infection, colonisation and sporulation by *Pseudocercospora macadamiae* on macadamia fruit. *Australas. Plant Pathol.* 38 (1):36–43.

Application methods of phosphonate to control *Phytophthora* pod rot and stem canker on cocoa

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INTRODUCTION

Stem canker and *Phytophthora* pod rot (PPR) or black pod caused by *Phytophthora palmivora* are among the most serious diseases of cocoa in Sulawesi, Indonesia, causing high yield losses for farmers. Potassium phosphonate (phosphite) has previously been demonstrated to effectively control canker and PPR in Papua New Guinea (1). To test the effectiveness of phosphonate in Sulawesi against *Phytophthora* diseases and to compare methods of application of the chemical, two experimental trials were conducted on cocoa farms in Sulawesi, Indonesia.

METHODS

1 The effect of trunk-injected phosphonate on stem canker and PPR in Ladongi, Southeast Sulawesi. Fifty 10 year-old hybrid cocoa trees were injected annually with 16 g a.i. phosphonate (Agrifos 600; Agrichem), fifty with water and fifty left untreated. For 4.5 years following the initial injection, trees were scored each month for canker severity and monitored for PPR and CPB incidence (% ripe pods affected). Treatments were compared by one-way ANOVA followed by either the Bonferroni or the Games-Howell post-hoc tests.

2 The effect of phosphonate on stem canker applied by three differing methods. In a blocked trial with four replicates, 2-year-old grafted cocoa trees naturally infected with *Phytophthora* stem canker were treated with phosphonate either by trunk injection, bark painting (combined with Pentrabark; Agrichem), or implants?, or left untreated and were then scored monthly (as in Experiment 1) for five months for canker lesion size.

RESULTS AND DISCUSSION

1 Trunk injection. Phosphonate injection cured stem canker within four months of the initial injection (Fig. 1). Over a 2.5 year period, phosphonate significantly decreased cumulative PPR incidence (Table 1), while PPR incidence did not differ between the untreated and water-injected trees indicating that the injection procedure itself did not influence these results. In both the control and phosphonate-treated trees, cycles of PPR infection occurred in the wet season with PPR incidence fluctuating from less than 30% to greater than 75% (data not shown). These might have been due to variations in rainfall, the regular removal of infected pods in the experiment or natural cycles of sporulation and infection. CPB incidence did not differ significantly between treatments (data not shown).

2 Phosphonate application. In a five-month period following the treatments the average canker lesion area remained the same or increased slightly in untreated trees, but decreased markedly in the trees treated with phosphonate, regardless of the method of application, from initial lesion areas of 50–100 cm², to 10–50 cm² after one month, and less than 10 cm² after five months. Since phosphonate effectively controls stem canker and decreases PPR in the long-term it provides a valuable option for the management of these diseases for cocoa smallholders.

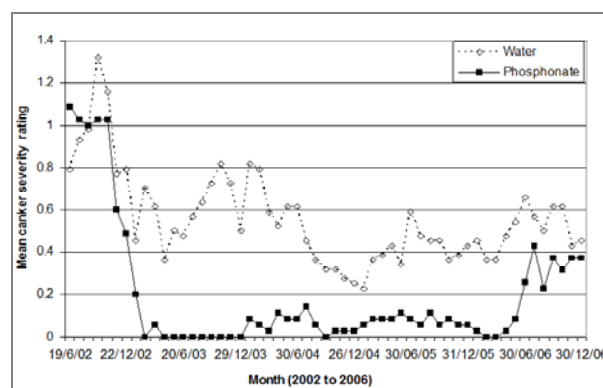


Figure 1. Mean canker scores for phosphonate-injected trees (solid line and squares) and water-injected (broken line, open diamond symbols) assessed from 2002 to 2006. Only trees that initially had canker infections are included. Trees were injected in June 2002, December 2002 and then every year until the end of 2006.

Table 1. Mean cumulative incidences of PPR in ripe pods from April 2004 to December 2006.

Treatment	Mean PPR (%)
Untreated	43.4 ± 1.9 ^a
Water-injected	40.2 ± 2.1 ^a
Phosphonate-injected	26.3 ± 1.8 ^b

Mean incidences were calculated from the total number of ripe pods and infested/infected pods harvested from each tree in the 2.5 years. Each treatment had 50 trees (replicates). Means (± SE) within columns followed by the same letter are not significantly different ($P < 0.05$).

ACKNOWLEDGEMENTS

The authors would like to thank Haji Sudirman and Les Nusa for providing cocoa trees on their farms for the trials and Agrichem, Queensland.

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Botrytis bunch rot control strategies in cool climate viticultural regions of Australia and New Zealand

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INTRODUCTION

Botrytis bunch rot can cause major losses in wine grapes, while negligible damage may occur in some seasons, even in the absence of control measures. We evaluated the effectiveness of different disease control strategies in five cool climate viticulture regions in the context of determining disease risk during the season (1) and economic outcomes for the grower.

MATERIALS AND METHODS

Standard measures of yield, weather, canopy density, bunch exposure, latent *Botrytis cinerea* incidence at pre-bunch closure (PBC), and botrytis development post-veraison were recorded at 23 sites in Southern Tasmania, Southern Victoria and New Zealand between 2006–08 (Table 1). Untreated plots and combinations of treatments including fungicide timing, canopy modification (leaf plucking and shoot thinning), and inoculum reduction by removing bunch trash, were used to generate a wide range of harvest botrytis severities.

RESULTS

The efficacy of early season (5%, 80% cap-fall), mid season (pea-size, PBC), late season (veraison, pre-harvest) fungicide applications and combination treatments varied considerably across the different trial sites and seasons. Only 8 out of 23 trials exceeded 3% botrytis severity at harvest (a level at which many wineries impose price penalties) on unsprayed vines. Fungicide applications reduced disease below the 3% threshold in only four of them (Table 1). Leaf plucking was as effective as the fungicide treatments in six New Zealand and one Victorian trial, but not in two other trials. Leaf removal combined with fungicide applications was the most effective treatment, reducing botrytis severity to below the 3% threshold even in trials where severity was >8% in the untreated plots. The experimental inoculum reduction treatment (bunch trash removal) did not reduce botrytis severity in these trials.

DISCUSSION

Assuming that 3% botrytis severity is a threshold level at which growers may suffer a price penalty on their crop, many of our trial sites did not reach the 3% severity threshold even when left untreated. Botrytis severity at harvest is strongly correlated with the length of the ripening period (1) and Auckland and Hawke's Bay were most prone to serious botrytis epidemics, followed by Marlborough and Southern Tasmania, with Victoria the lowest risk. These results highlight the need for benefit-cost analyses, as the full cost of a spray program (fuel, pesticide, labour, water, etc) may not always be recouped, particularly in drier viticultural regions like Victoria.

Leaf plucking was as effective as a complete fungicide program at sites with dense canopies. The combined effects of leaf removal and fungicide application gave the lowest levels of botrytis, but risk of sunburn and effects on fruit quality are also important when considering leaf plucking for botrytis control.

Tactical decisions about the need for individual fungicide sprays or canopy management actions depend on the season, canopy density and bunch exposure, and underlying risk for the region. The data collected in these trials is being used to develop a botrytis risk model (1) to assist growers in making decisions for botrytis management in future.

Table 1. Treatment effects on botrytis severity at harvest for trials conducted 2006–08 in Australia and New Zealand.

Year	Location and Variety ¹	Mean botrytis severity at harvest (%)			
		Control	Spray program ²	Leaf pluck	Spray +leaf pluck
06–07	NZ (A) - SB	25.3	7.7 (mid)	-	-
"	NZ (HB) - SB	4.5	2.1	2.6	0.3
"	NZ (HB) - CH	9.6	5.9 ns	2.2	1.7
"	NZ (M) - SB1	0.2	0.1 ns(early)	-	-
"	NZ (M) - SB2	0.6	-	-	-
"	Tas - SB	2.5	0.9 (mid)	-	-
"	Tas - R	0.6	0.8 ns	-	-
"	Vic - CH1	0.0	0.0 ns	-	-
"	Vic - SB	0.1	0.0 ns	-	-
"	Vic - CH2	0.0	0.0 ns	-	-
07–08	NZ (A) - SB	1.6	0.1 (early)	0.7	0.3
"	NZ (HB) - SB	8.4	3.5	3.4	0.4
"	NZ (HB) - CH	9.5	1.9	1.9	0.3
"	NZ (M) - SB1	2.1	1.5 (mid/early)	1.1	0.2
"	NZ (M) - SB2	0.8	-	-	-
"	NZ (M) - SB3	5.3	0.6	-	-
"	Tas - SB	2.7	1.6 ns (mid)	2.2	1.9
"	Tas - R1	6.2	2.6 (mid)	-	-
"	Tas - R2	1.9	0.8 ns (mid)	-	-
"	Tas - CH	3.6	2.8 (late)	-	-
"	Vic - CH1	2.0	0.0	2.1	0.0
"	Vic - SB	2.3	0.1	0.4	0.6
"	Vic - CH2	0.8	0.0	0.6	0.0

¹ M=Marlborough, HB=Hawke's Bay, A=Auckland, SB=Sauvignon Blanc, R=Riesling, CH=Chardonnay

² Full fungicide program or the most effective fungicide timing (shown in brackets). ns—fungicide treatments not significantly different to untreated at $P \leq 0.05$

ACKNOWLEDGEMENTS

GWRDC, New Zealand Winegrowers and our respective agencies for funding this research.

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Development of a soil DNA extraction and quantitative PCR method for detecting two *Cylindrocarpon* species in soil

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INTRODUCTION

Cylindrocarpon black foot disease has been identified worldwide as a common cause of vine death in nurseries and in young vineyards (1), especially in sites converted from orchards or replanted from grapes. This indicates that the existing soil conditions may have contributed to the disease, although very little information is available regarding the survival of the pathogens in soil. In New Zealand, the three *Cylindrocarpon* species equally responsible for black foot disease on grapevines are *C. destructans*, *C. macrodidymum* and *C. liriodendri* (1). Recently, quantitative PCR (qPCR) has begun to supersede soil dilution plating as a method for precise determination of soil inoculum levels (2). In this research program, qPCR was optimised to test large soil samples for presence of two *Cylindrocarpon* species.

MATERIALS AND METHODS

Fungal isolates. The Lincoln University Culture Collection provided isolates of the three *Cylindrocarpon* species, which had been obtained from symptomatic New Zealand grapevines. Single spore colonies of 10 randomly selected isolates per species were grown on potato dextrose agar (PDA) at 20°C in the dark.

Soil Infestation. A mixed conidium suspension (10^6 conidia /mL) was obtained for each species using three isolates. They were used to infest 5 L pots of soil, three replicates per species, with a final concentration of 10^5 conidia per gram of soil. Controls were treated with a similar quantity of water. The 5 L pots were sunk into, and level with, the ground in the Lincoln University Vineyard. Two 15 g soil samples were taken from each pot for DNA extraction at 0, 1, 2, 3 and 6 weeks after set up. All three species were inoculated into each pot to ensure detection specificity

DNA extraction. For pure culture extraction, a small plug of mycelium was transferred from a 5 d old PDA culture to potato dextrose broth (PDB) and incubated for 7 d at room temperature, in 12 h light/ dark. The mycelium was harvested and stored at -80°C until DNA extraction using a PureGene DNA extraction kit (Qiagen). Spectrophotometry was used to quantify the genomic DNA. DNA was also extracted from 10^6 conidia of each isolate in a similar way.

For DNA extraction from soil samples, 10 g of soil was placed in a 250 mL bottle with 45 mL water containing 0.01% agar. The bottles were shaken for 10 min and left to stand for 10 min. The supernatant was put into two 15 mL tubes, and centrifuged at 4000 x g for 15 min. The pellets were combined and centrifuged again, with the final pellet being used for DNA extraction using a PowerSoil kit (MO BIO Laboratories Inc.). Genomic DNA quality was assessed by visualisation on a 1% agarose gel.

PCR amplification. Species specific primers for the β - tubulin region of *C. macrodidymum* and *C. liriodendri*, donated by Dr Lizel Mostert (University of Stellenbosch, South Africa), were used in qPCR with SYBR green chemistry and a *Rox* internal standard on an ABI Prism 7700 sequence detector. After the qPCR, the products were separated by electrophoresis on a 1.5% agarose gel and visualised under UV light.

RESULTS

The primer pairs were specific for each of the two species and no cross reactivity was observed. They produced a 300 bp and 197 bp product for *C. macrodidymum* and *C. liriodendri*, respectively. They were also suitable for a range of genetically diverse isolates. Each of the primers was able to detect as little as 30 pg DNA in a standard PCR and 3 pg DNA in a nested PCR. The qPCR could detect pure DNA at the same level as the nested PCR. For spore suspensions, the qPCR was able to detect as little as 100 spores in soil. The time course experiment showed that for each species, less than half of the nuclei remained 1 week after infesting the soil with conidia. However, at this time, the DNA could still be visualised on agarose gels of the qPCR products (Fig 1). After 2, 3 and 6 weeks, the DNA could not be detected.

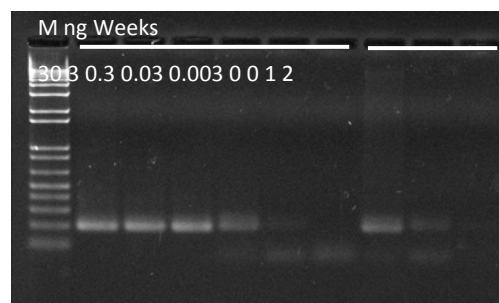


Figure 1. 1.5% agarose gel of products amplified during qPCR with the primers specific for *C. liriodendri*.

DISCUSSION

The results show that the species specific primers used in a qPCR system could detect as little as 3 pg of pure DNA, which is equivalent to 30 *Cylindrocarpon* macroconidia. When spores were recovered from soil a sensitivity of 100 spores was achieved. Following soil inoculation, DNA of fewer than 50% of the conidium nuclei in the soil was detected after 1 week. Additional unpublished data has indicated that in the soil environment, the 4-celled conidia were often converted to 2 chlamydospores (uninucleate). It is possible that the >50% decrease observed is a reflection of that conversion, rather than the death of conidia. Research is continuing to investigate the population dynamics of these *Cylindrocarpon* species in soil. Future research will include the development of *C. destructans* specific primers.

ACKNOWLEDGEMENTS

We gratefully acknowledge Lizel Mostert for providing the primers sequences and Winegrowers New Zealand and TECNZ for funding this project.

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Why Australia needs a coordinated national diagnostic system

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INTRODUCTION

The accurate and rapid diagnosis of plant pests and diseases underpins all management activities aimed at preventing the establishment of exotic pests and is a critical component of surveillance activities.

Plant pest diagnostic services also provide the foundation of endemic pest management through the timely identification of plant pests, if left uncontrolled, can disrupt agricultural plant production and restrict market access for agricultural produce. Rapid identification also supports quarantine processes, such as maintaining pest free areas that allow access to domestic and international markets.

Diagnostic services are delivered by a range of agencies across a dispersed geography and climate range. The majority of diagnostic services are provided by state agencies with some being delivered by the Australian Government, commercial diagnostic laboratories, CSIRO, and the Universities (1). Services are provided on an *ad-hoc*, commercial and nationally coordinated basis as required. Diagnostic operations are often performed in conjunction with collaborative research activities.

The development of a national diagnostic system for plant pests and diseases has been the subject of discussion for over 10 years. A number of reviews have been conducted of the national capability (1, 2) and issues of standards and accreditation have been addressed by the Subcommittee on Plant Health Diagnostic Standards (SPHDS) (3). More recently SPHDS has been given the responsibility to develop a National Diagnostic Strategy for plant health. This strategy will complement the National Plant Health Strategy that will be released this year.

This paper reports on the activities of four diagnostic laboratories, their findings and implications for national biosecurity.

METHODS AND RESULTS

Data was collected from state government diagnostic laboratories in Tasmania, South Australia, Victoria and NSW. Data was also provided by the Office of the Chief Plant Protection Officer (OCCPO).

Between January 2006 and May 2009, 45 new pest or diseases have been brought to the attention of the OCPPO and have warranted action by the Consultative Committee on Exotic Plant Pests. Of these 16 were fungi, 13 were invertebrates, 11 were virus or phytoplasmas and 5 were bacteria.

In the same time the Victorian DPI diagnostic laboratory conducted investigations into 133 suspect emergency plant pests. Some of these investigations arose from detections interstate and consequent national surveillance activities. Of

interest is that 43% of these investigations arose from ad hoc samples submitted to the laboratory from industry. Most of these were ornamentals. The diagnostic investigations revealed 15 new hosts of pathogens already present in Australia, three new pathogen records for Victoria, seven new pathogen records for Australia and one new undescribed species.

Data from the other states proved difficult to obtain and to analyse. Diagnostic activity was not centrally coordinated and details were not easily accessed due to a lack of readily searchable databases. South Australian laboratories conducted in excess of 90 investigations into suspect emergency plant pest, NSW 88, Queensland 63 and Tasmania 20.

The numbers of investigations conducted by DPI Vic have increased from 16 in 2006 to 64 in 2008. Similarly in NSW numbers increased from 19 in 2006 to 25 in the first five months of 2009. This is in part due to a better understanding by the diagnostic laboratory of reporting obligations under the Emergency Plant Pest Response Deed (4).

DISCUSSION

The data presented here demonstrates the increasing level of activity and importance of diagnostic activity nationally. It also highlights the benefits of a central coordination of diagnostic services within an agency. Those agencies without a centrally coordinated diagnostic service find it difficult to ascertain the level of diagnostic activity that underpins their states biosecurity. This shortcoming will be overcome as Australia works towards developing a National Diagnostic System.

ACKNOWLEDGEMENTS

To all the diagnosticians in Australia who have contributed to the data presented here.

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Bananas in Carnarvon—good news for growers in survey for quarantine plant pests and pathogens

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INTRODUCTION

The banana industry in Carnarvon, Western Australia, is unique among other Australian banana growing areas. While bananas are typically grown in tropical to sub-tropical regions where rainfall is abundant, Carnarvon has an arid-desert climate and growers depend on year round irrigation. Under these unique conditions, plant pests and pathogens common to other Australian banana growing areas may not be successful. However, pathogens and pests more suited to local conditions could potentially thrive. A survey to identify potential quarantine risks and exotic viruses, bacteria, fungi, insects and nematodes was initiated by HortGuard[®] and the Carnarvon Banana Producers Committee.

METHODS

From the approx. 55 banana growing properties in the Carnarvon area, 15 were selected for assessment based on a range in production and management practices, yields and years in production. Survey activities comprised three main areas, each with specific sampling methods.

Nematodes. From each property, soil and roots were collected from ten trees using methods adapted from Pattison *et al.* (1). Sample trees were chosen randomly within older blocks where nematode numbers were likely to be higher. Roots were examined for symptoms. Nematodes were extracted from roots and soil in a mist chamber over 5 days, then quantified and identified.

Virus, bacteria and fungi. A minimum of 100 plants per property were inspected. Leaves, pseudostems, suckers and bunches were visually assessed for symptoms of disease, mechanical and physiological damage. Symptoms were described in terms of type, quantity and severity. The most severely infected leaves were incubated in moist trays under natural light for 2–7 days. For identification, fruiting bodies on leaves were examined microscopically, on half strength Potato Dextrose Agar and on Water Agar. As no symptoms were observed for viruses, further testing was not conducted.

Invertebrates. At each property 2–4 blocks of different aged plants were assessed using sweep netting and direct observation. Sweep net samples were collected from approx. 200 sweeps of the main canopy and from 100x10 minute sweeps near the plantation floor. Direct observation (10x hand lens) focused on invertebrates found on leaves, fruit, flowers and the plantation floor.

RESULTS AND DISCUSSION

Nematodes. No nematodes of quarantine significance were identified. Root Knot Nematode (RKN, *Meloidogyne* sp.) and Spiral Nematode (*Helicotylenchus multincinctus*) were identified from both the roots and soil (Table 1). Spiral Nematode and RKN were present in all samples. Root Lesion Nematode (*Pratylenchus* sp.) was identified from the roots, but not the soil in only one sample (2.7/g dry root). Root symptoms of both RKN and Spiral Nematode were observed. Typical symptoms of Burrowing Nematode (*Radopholus similis*) were absent, and this species was not identified from any sample.

These nematode levels would not be regarded as a production constraint in tropical areas. In Carnarvon, where plants did not have well developed root systems, it is possible that Spiral and Root Knot Nematodes may have a greater impact (T. Pattison, pers. comm., 2009). In tropical areas, Spiral Nematode is of secondary importance

to Burrowing Nematode. However, in areas where temperature and rainfall conditions are limiting, *R. similis* is rare, and *H. multincinctus* is the major nematode problem which can cause severe damage and decline in bananas.

Table 1. Spiral and Root Knot Nematode (RKN) densities extracted from roots and soil.

Property	Nematodes/g dry root		Nematodes/200g dry soil	
	Spiral	RKN	Spiral	RKN
1	473.0	25.9	533.1	58.2
2	0	56.6	8.5	357.6
3	162.2	67.4	183.7	164.4
4	142.9	32.0	1418.9	54.2
5	638.9	9.7	892.4	29.6
6	530.4	83.1	236.5	19.4
7	254.8	22.8	245.6	18.9
8	149.5	39.0	571.6	89.1
9	458.1	21.7	577.2	25.5
10	344.5	22.1	1141.9	26.8
11	38.4	279.9	59.4	80.2
12	675.5	1.3	640.6	7.7
13	415.0	188.3	404.7	11.4
14	433.8	3.8	450.1	8.4
15	12.5	200.9	0	167.3

Virus, bacteria and fungi. No diseases of quarantine significance were identified. From the 20 samples collected, secondary fungal pathogens were identified from 14, including *Alternaria* sp., *Stemphyllium* sp., *Penicillium* sp. and *Aspergillus* sp. *Colletotrichum* sp. was identified from leaf spots on one sample. *Pleospora herbarum* was identified from one sample of a leaf spot with dark margin and bleached centre. *P. herbarum* is a cosmopolitan fungus that is occasionally a weak parasite known to cause leaf spots.

Invertebrates. No pests of quarantine significance were identified. Pest levels were low over the entire area. Seventy per cent of the surveyed properties use biocontrol agents, which may account for the low pest numbers. Large numbers of spiders, which are beneficial in reducing pest invertebrates, were found on all properties. These included typical wheel-web spiders (Araneidae) such as *Eriophora* spp. as well as Sparassidae, Lycosidae and Salticidae.

CONCLUSION

No exotic pests or diseases were identified. Only low levels of fungal pathogens and invertebrate pests were detected. The unique environmental conditions of Carnarvon, as well as its isolation from other banana growing areas, may contribute to this finding. Although Burrowing Nematode (*R. similis*) was not detected, Spiral and Root Knot Nematodes may pose a potential production constraint to bananas in this area.

ACKNOWLEDGEMENTS

Thanks to the Carnarvon Banana HortGuard[®] Committee and the APC Carnarvon Banana Producers Committee for supporting and funding this work. D. Parr and S. Lawson assisted with sample collection. H. Hunter, X. Zhang, L. De Brincat, C. Wang, M. You and N. Eyres processed, and identified samples in the laboratory.

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Can investment in building up soil organic carbon lead to disease suppression in vegetable crops?

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INTRODUCTION

Over the past few years, there has been increasing recognition of soil as an important non-renewable asset that needs to be managed well and looked after. Practices to improve soil health are increasingly being recommended such as building up soil organic carbon by the addition of green manures, animal manures, organic mulches, composts, etc.

Soilborne diseases have traditionally been controlled with the use of chemicals such as fumigants and fungicides. These options are being withdrawn worldwide for human health and environmental reasons. Alternative methods of control such as the use of biofumigant crops and strategic nutrient application are now being used more widely by industries. However, what is the penalty cost of using these methods when conditions are unfavourable for disease? Can improvements in soil health such as building up organic carbon be used to create disease suppressive conditions on vegetable farms?

MATERIALS AND METHODS

Trials were set up to examine the impact of disease management practices and soil organic amendments on crop productivity and profitability at two vegetable farms in southern Victoria.

At site 1, with a history of clubroot of brassica (*Plasmodiophora brassicae*), chemical fumigation, biofumigation, fungicides, nitrate and slow release ammonium fertilisers and organic soil conditioners (chicken manures, silage and composted green waste) were applied to broccoli grown during winter when conditions were unfavourable for disease.

Long-term trials were also established at both sites to compare the effect of organic amendments with the standard practice of metham fumigation on crop yield, profitability and soil health characteristics. Treatments were applied in autumn and spring of 2008. Crop rotations included lettuce, broccoli, endive, leeks and celery.

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 measured but data analysis is still under way.

RESULTS AND DISCUSSION

Tailored site-specific nutrient applications were able to provide equivalent yields (Figure 1) and profit as soil fumigation, with potentially better benefits to the environment. The biofumigants, Voom and Fumifert, and composted organic mulch gave yields equivalent to standard grower practice. Fluazinam, a pesticide registered for clubroot control, resulted in lower yields than standard grower practice due to its tendency to cause a delay in crop maturity.

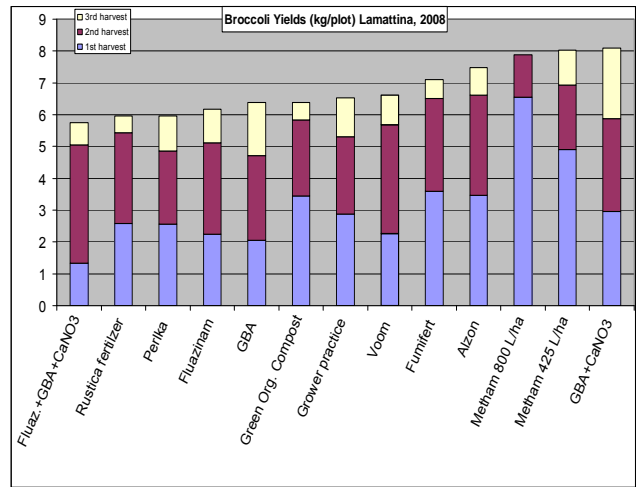


Figure 1. Short term study: Marketable yield of broccoli (3 harvests) grown with different soil treatments in winter 2008 at Boneo, Victoria, LSD = 1.24.

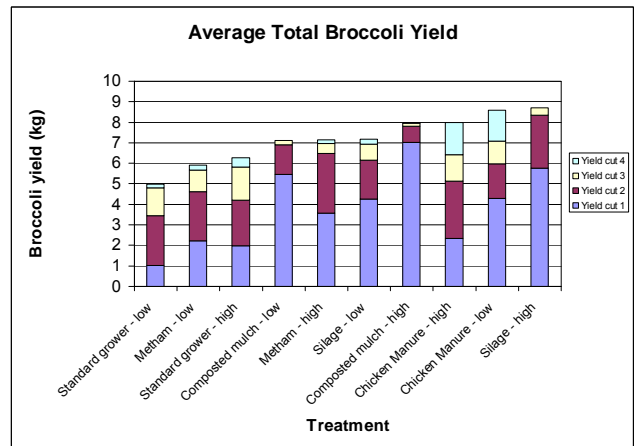


Figure 2. Long term study: Marketable yield of broccoli grown with different organic amendments during winter 2008.

Organic amendments, although producing moderate yields (Figure 1), reduced grower profit in the short term study, but increased yields and maintained profit in the long term study (Figure 2). The trials also confirmed better water and nutrient use efficiency and biological indicators are showing an increase in biological activity which leads to better soil resilience and possible disease suppression. Current and future trials have been set up to evaluate the long term effect of repeated treatments, particularly with respect to disease suppression. A cost/benefit model is also being developed to help growers determine the benefits of soil health for different crop production systems.

ACKNOWLEDGEMENTS

Horticulture Australia Ltd, DPI Victoria and the Vegetable Industry funded this research.

Evaluation of soil health indicators in the vegetable industry of temperate Australia

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INTRODUCTION

Soil health benchmarking trials commenced in the temperate vegetable industry in 2007 as part of a national program to better understand soil health and its impact on vegetable production efficiency and soilborne diseases.

The aim of the benchmarking trials was to identify soil biological, physical and chemical tests that could be used to detect changes in soil caused by various farm management practices. Ultimately, the study aims to use these indicators to identify good soil health strategies that suppress soilborne pathogens.

MATERIALS AND METHODS

In 2007, 14 commercial production sites and three non-production ("control") sites were selected at two vegetable farms on sandy soil at Cranbourne, Victoria.

Sites that were at different stages of production (table 1) were compared with each other and with non-production sites. A suite of biological, chemical and physical tests were evaluated for their robustness as indicators of soil health under a range of farm practices (1).

Table 1. Site selection for benchmarking studies

Site name	Crop stage at sampling
TS1	6 week spinach
TS2	Fallow
TS4	Celery transplant
PS1, PS3A	Leeks residue incorporated
PS2, PS3B	Leeks mature
PS4	Kohl rabi residue incorporated
TS33a, TS43a,c	Fumigated, lettuce transplant
TS33b, TS43b,d	Lettuce transplant
PS5, TS3, TSNC	Non-production

RESULTS AND DISCUSSION

Biological tests. Populations of free-living nematodes were quantified as an indicator of a site's previous history and potential resilience following disturbance. Populations and community structure of free-living nematodes were affected by soil disturbances such as tillage, fumigation, or residue incorporation, but tended to recover over time. These data indicate that although tillage, fumigation and crop residue management can stimulate flushes of bacterial colonisation, populations of microbial organisms may stabilise during crop growth. This was more evident for crops of longer (leeks) rather than shorter (lettuce) duration. These observations are important when considering the resilience of soils and long term sustainability of vegetable production.

Chemical tests. Available phosphorus was always higher in production compared with non-production sites, irrespective of cropping history (Figure 1). Combined with wide-ranging levels of potassium and sulphur measured in many cropped sites, these results suggest inappropriate fertiliser application may be common in vegetable production. The impact of this on control of soilborne diseases could be important and will be considered in future studies.

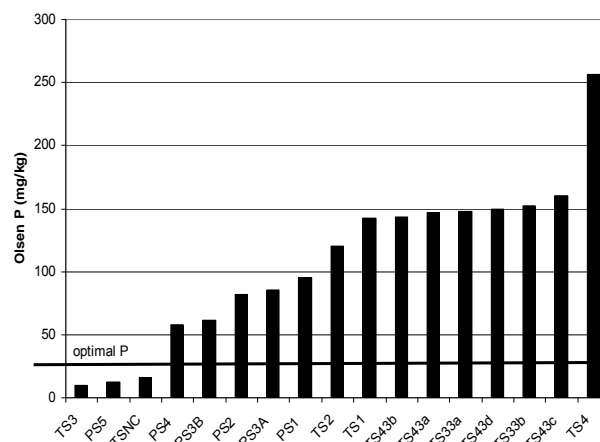


Figure 1. Phosphorus levels were higher than required for vegetable production at cropped sites compared with non-production sites, irrespective of cropping history.

CONCLUSION

On the sandy Cranbourne soils, nematode communities responded rapidly to changing soil conditions caused by farm practices and are a good indicator of past soil management, and potentially a good indicator of soil resilience and soil health. Some chemical measures such as available phosphorus, potassium and sulphur were useful in identifying poor fertiliser management and therefore poor soil health practices.

Additional chemical and biological measures such as nitrogen, carbon and soil-borne disease thresholds are being evaluated in current field trials to identify indicators that show how altered farm management can improve suppression of soilborne diseases.

The indicators of soil health that prove to be robust predictors of the relationship between on-farm practices, crop yield and soil health will be further developed so that growers can improve nutrient, water and disease management on farm. The information will become part of a national database which will enable growers to better manage farm inputs and improve the sustainability and soil health of the vegetable industry in temperate Australia.

ACKNOWLEDGEMENTS

This work was funded by Horticulture Australia Ltd, the Victorian Vegetable Industry and the Victorian DPI. We thank the vegetable farmers who provided land and resources to conduct these trials.

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Rhizoctonia AG2.1 and AG3 in soil—competition or synergism?

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INTRODUCTION

Rhizoctonia solani causes stem and stolon canker, which delay and reduce emergence, as well as black scurf on tubers. AG2.1 and AG3 are the most dominant anastomosis groups isolated from potato plants (1). The interactions between AG2.1 and 3 have not been fully examined. Strains within the one species that co-occur in a habitat (soil) and may have similar resource requirements may be expected to compete with each other. Competition between fungi has been categorised as either 1) colonisation of unoccupied habitat or 2) colonisation of habitat that is already occupied (2). This study used radish as a model system 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: 1—1 plate fungal mycelium, 0.5—half plate fungal mycelium, 0.25 quarter plate fungal mycelium per 8 kg soil (Table 1). Nine radish seeds were planted per pot with 5 replicate pots, and grown at 17°C in a growth room (cool) or 27°C in the glasshouse (warm). Emergence and pruning of the radish seedlings was assessed weekly for 5 weeks. Plants were harvested and assessed for yield, after 4 weeks (warm conditions), or 8 weeks (cool conditions).

Table 1. Soil inoculation combinations and rates (plates/8kg soil).

Treatment	AG2.1	AG3
1	1	0
2	0	1
3	1	1
4	0.5	0
5	0	0.5
6	0.5	0.5
7	0.5	1
8	1	0.5
9	0.25	1
10	1	0.25
11	0.25	0
12	0	0.25
13	0.25	0.25
14	0	0

RESULTS AND DISCUSSION

AG2.1 by itself only affected emergence at the highest rate, under cool conditions (Figure 1). AG3 by itself had no effect under either cool or warm conditions. At the highest rate of AG2.1, in combination with AG3 at any concentration significantly reduced radish seedling emergence in both cool and warm conditions. Under cool conditions, AG2.1 is more severe in combination with AG3 at reducing radish seedling emergence than under warmer conditions. However, with lower rates of AG2.1, there was little effect on emergence. Therefore, when AG2.1 is found in high concentrations in soil the addition of AG3 at any rate decreases the level of emergence under both cool and warm conditions suggesting a synergistic relationship between the AGs to cause disease.

There was a good negative relationship between AG2.1 soil inoculum treatments and radish emergence ($R^2 = 0.9$).

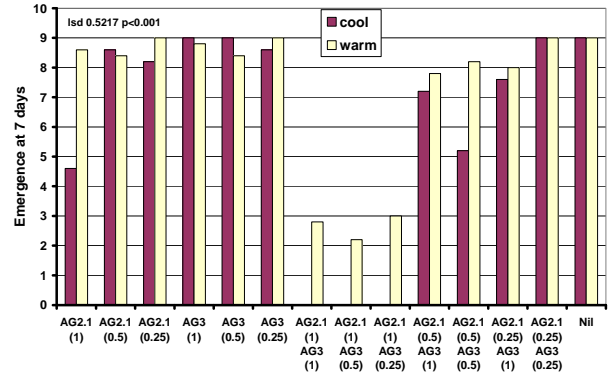


Figure 1. The effect of soil inoculation treatments on radish seedling emergence after 7 days.

There may be multiple explanations for observed changes and difference in the frequency at which different strains of the same species may colonise soil overtime. The next stage of this research will be to use these treatments and growing conditions to monitor soil colonisation overtime and study disease expression in potato plants.

ACKNOWLEDGEMENTS

This project was facilitated by HAL in partnership with the processing potato industry, funded by the processing potato levy and voluntary contributions from industry partners. The Australian Government provided matched funding for HAL’s R&D activities. DPI Victoria contributed funding to this project.

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Towards universal detection of *Luteoviridae*

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INTRODUCTION

Luteoviridae is a plant virus family of 26 species which causes yield losses in cereals, potatoes and other economically important crops worldwide. Accurate detection and fast identification are important components of controlling the spread of luteoviruses and reducing yield losses.

PCR based detection is often the method of choice for *Luteoviridae* as it is more sensitive than serological methods [1] which frequently fail to detect infection due to the low concentration of luteoviruses in plants. Since the currently available luteovirus primers are mostly species specific and work under different PCR conditions, universal primers are desirable.

Following PCR amplification sequencing may be required to confirm virus identity, often resulting in several days delay. A possible alternative is the use of melt curve analysis (MCA) which requires less time than sequencing and can be performed in most real-time PCR equipment. MCA is already used for identification of some animal and plant viruses.

We tested 7 primers designed to target the most conserved regions of the *Luteoviridae* genomes and which possess high homology to over 75% of *Luteoviridae* species. We also tested the suitability of MCA in identification of *Luteoviridae* species.

MATERIALS AND METHODS

Virus isolates. Thirty luteovirus isolates representing 15 species were obtained from New Zealand and overseas (either as infected freeze dried plant material or as cDNA). These included all 5 species from the *Luteovirus* genus, 8 of the 9 species from the *Polerovirus* genus (except CYDV-RPS), PEMV-1 (the only species from the *Enamovirus* genus) and CtRLV which is not assigned to a genus.

PCR. Reactions were performed using three combinations of primers (7 primers in total):

1. C1F1/C1F2/C1R1/C1R2 (129bp and 148bp amplicons)
2. C2F1/C2F2/C2R3 (68bp amplicon)
3. C1R1/C2R3 (75bp amplicon)

All primers have a low level of degeneracy and amplify a fragment from the coat protein gene. Amplification products were sequenced to confirm their identity.

Melt Curve Analysis. Real-time PCR was performed using the same three primer combinations as above, SYBR GreenER I fluorescent dye and an ABI PRISM[®] 7000 real-time cycler. The melting of amplification products was performed at a rate of 0.2°C/min.

RESULTS AND DISCUSSION

Between them the three combinations of primers detected all 13 of the the *Luteovirus* and *Polerovirus* species tested:

1. C2F1/C2F2/C2R3 primers detected nine species (Fig.1).
2. C1F1/C1F2/C1R1/C1R2 primers detected BYDV-PAV, BYDV-MAV and BYDV-PAS.

3. C1F1/C2R3 detected ScYLV species.

None of the primer combinations detected PEMV-1 or CtRLV.

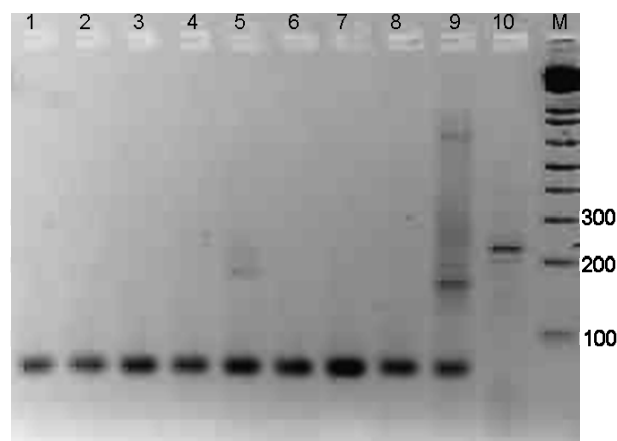


Figure 1. Amplification of *Luteoviridae* with primers C2F1, C2F2 and C2R3. Lanes: 1 – BWYV, 2 – BMYV, 3 – BCIV, 4 – BLRV, 5 – PLRV, 6 – TuYV, 7 – CABYV, 8 – CYDV-RPV, 9 – SbDV, 10 – Negative (water), M – 1kb Ladder (NEB).'

The primers are homologous to 6 other *Luteoviridae* species (SPLSV, BYDV-SGV, BYDV-GPV, BYDV-RMV, GRAV and TVDV) but amplification of those species is yet to be tested.

Melt curve analysis showed that at least 5 *Luteoviridae* species can potentially be distinguished by different melt temperatures; but exact temperatures are yet to be determined.

ACKNOWLEDGMENTS

We acknowledge Dave Saul and Karin Farreyrol (Univ. Auckland) for the original primer design. We thank J.Fletcher, C.Delmiglio, O.LeMaire, M.Stevens, B.Wintermantel, S.Sauntally, T.van Antwerpen and S.Fuentes for kindly supplying virus isolates and MAF Bio-Security New Zealand, in particular J.Tang, for technical assistance. This work was supported by MAF Biosecurity New Zealand and Better Border Biosecurity.

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Massive parallel sequencing of small RNAs to identify plant viruses and virus-induced small RNAs

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INTRODUCTION

Small RNAs are short (20–25 nt) RNAs that can guide the degradation of target mRNA or alternatively inhibit their translation. The net result of either process is the lack of functionality of the target RNA. In plants, small RNAs are generated from two distinct sources: the plant genome or infecting viruses¹. The intrinsic small RNAs encoded by a plant are mainly composed of microRNAs that are processed from longer RNA transcripts and target other plant-encoded mRNAs. Small interfering (si) RNAs are derived from an infecting virus and target that same virus RNA for degradation, thus forming the basis of a highly malleable, sequence-specific defence mechanism.

Recent advances in sequencing technologies now permit the economic identification of millions of RNA sequences from a single sample of plant tissue. Knowledge of the siRNA sequences in a plant infected with an unknown virus provides a potential tool for identification of the virus from which the sequences are derived. This process is aimed at use at the border by Biosecurity New Zealand.

MATERIALS AND METHODS

Plant and virus samples. *Nicotiana occidentalis* plants grown at 20°C with 10 hours low light per day were either mock-inoculated or inoculated from leaf samples infected with one of four known viruses or from six plants infected with unknown viruses. Small RNAs were isolated and sequenced using the Genome Analyser (Illumina®) at the Allan Wilson Centre Genome Sequencing Service.

Bioinformatic programs. Data were matched to known targets using ELAND (Illumina®), or formed into contiguous sequences using Velvet² or Edena³ before matching to virus sequences in publicly available sequence databases using Basic Local Alignment Search Tool (BLAST)⁴.

RESULTS AND DISCUSSION

We have sequenced small RNAs from both uninfected and virus infected samples, resulting in over 22 million sequences (~500,000 unique sequences). The unique sequences present in uninfected tissue were subtracted *in silico* from the infected sequence pool to identify the small RNAs specific to uninfected tissue and those common to both uninfected and virus-infected tissues (Figure 1). The remaining sequences that were present only in the virus-infected tissue were then used either to match to a known infecting virus (Figure 2) or to develop a method to identify unknown virus infectious agents also present in the tissue. To increase search specificity, contiguous sequences were generated before BLAST analyses.

Subtraction *in silico* of the infecting virus siRNAs from the sequences found only in virus-infected plants left a large pool of small RNAs (~240,000 unique sequences) that were (presumably) not of viral origin. The identity of these novel plant-derived small RNA sequences present in response to virus infection is being investigated and will be discussed.

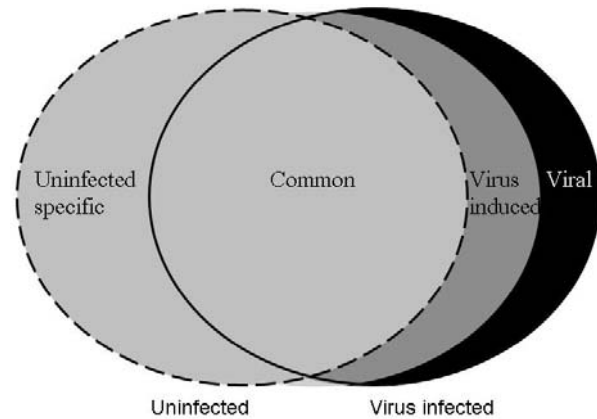


Figure 1. Venn diagram illustrating the pools of small RNA sequences derived from uninfected and infected tissue.

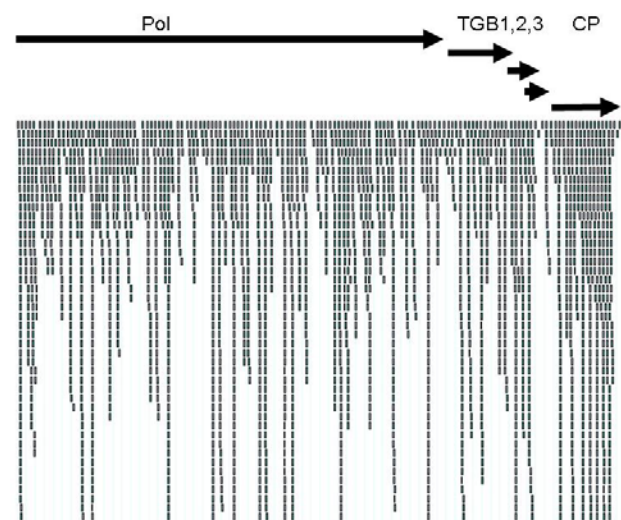


Figure 2. Alignment of small interfering RNAs to the genome of a potexvirus. The potexvirus genome (five arrows representing open reading frames) is aligned with the ~4,000 unique, cognate siRNA sequences (dashes).

ACKNOWLEDGEMENTS

This research is funded by the New Zealand Foundation for Research, Science and Technology (C06X0710).

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Chickpea chlorotic stunt virus, an important virus of cool-season food legumes in Asia and North Africa and potentially in Australia

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INTRODUCTION

Chickpea chlorotic stunt virus (CpCSV), a proposed new member of the genus *Polerovirus* (family *Luteoviridae*) was first described in Ethiopia in 2006 (1) and has since been reported from Eritrea (4), Syria (3), Egypt, Morocco and Sudan (2). It naturally infects many legume crops (e.g., chickpea, lentil, field pea, faba bean) as well as some leguminous weeds and four wild non-legume plant species (1, 2, 3, 4). Typical symptoms of CpCSV-infected plants are leaf rolling, yellowing and stunting (Figure 1-A). CpCSV is a phloem-limited virus that is present in very low concentrations and transmitted only by aphids (*Aphis craccivora* Koch.) in a persistent manner (1, 3).

This study reports the use of a few monoclonal antibodies to CpCSV (1, 2) for detecting different CpCSV isolates from 8 countries in Asia and North Africa.

MATERIALS AND METHODS

Sample collections, diagnostic techniques and reagents used—A total of 3265 food legume samples showing yellowing/stunting symptoms were collected from 8 countries (Azerbaijan, China, Eritrea, Ethiopia, Lebanon, Syria, Tunisia and Yemen) and tested for the presence of CpCSV using the following three mixtures of CpCSV monoclonal antibodies (MAbs) in tissue-blot immunoassay (TBIA): M-I = 1-1G5 + 1-3H4 + 1-4B12; M-II = 5-2B8 + 5-3D5; and M-III = 5-5B8 (1, 2). In addition, over 500 TBIA blots from chickpea, faba bean and field peas collected in northern NSW, Australia, for different types of symptoms were processed with the CpCSV MAbs.

RESULTS AND DISCUSSION

Figure 1-B shows how CpCSV is detected in infected plants by using TBIA.

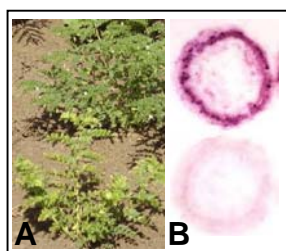


Figure 1. (A) Symptoms produced on a chickpea plant infected with chickpea chlorotic stunt virus (CpCSV); (B) TBIA detection of CpCSV in a stem blot from an infected chickpea plant (top) as compared to that from a non-infected plant (bottom).

Table 1 summarises the reactions of the MAbs with the samples from 8 countries in Asia and North Africa. Results obtained placed the tested samples in four groups: group A comprised 1218 samples (from Azerbaijan, Lebanon, Syria, Tunisia and Yemen) reacting with MAbs M-II and M-III; group B contained 254 samples (from Azerbaijan, China, Eritrea, Ethiopia and Tunisia) reacting only with MAb M-I; group C included 77 samples (from Azerbaijan and Ethiopia) reacting with MAbs M-I and M-II; and group D consisted of 38 samples (from Ethiopia and Tunisia) reacting only with MAb M-II. The presence of CpCSV was confirmed in a representative number of samples from each group and country by RT-PCR using specific primer sets. This is the first record of CpCSV in Azerbaijan, China, Lebanon, Tunisia and Yemen.

Testing of the Australian samples gave CpCSV-positive reactions for several chickpea, faba bean and field pea samples, which tested negative to antisera specific for other luteoviruses. These findings require reconfirmation, but are an indication that CpCSV (or a closely related non-described virus) may be present in Australia. Sequence analysis of RT-PCR amplicons is in progress and will shed more light on the relatedness among the aforementioned groups and to the two major CpCSV strains proposed by Abraham *et al.* (2).

Table 1. TBIA reactions of different luteovirus isolates with three groups of monoclonal antibodies raised against CpCSV

Country/Crop	No. of samples tested	No. of TBIA-positive samples	TBIA reaction with different CpCSV MAbs*		
			M-I	M-II	M-III
Eritrea					
Chickpea	211	32	+	-	-
Tunisia					
Chickpea	711	6	+	-	-
		8	-	+	-
		335	-	+	+
Faba bean	127	8	-	+	+
Azerbaijan					
Chickpea	320	11	-	+	+
		2	+	+	-
		153	+	-	-
		1	+	-	-
Lentil	86	1	+	-	-
Ethiopia					
Chickpea	129	70	+	+	-
		29	-	+	-
		1	+	-	-
Lentil	9	5	+	+	-
		1	-	+	-
Faba bean	190	4	+	-	-
Fenugreek	80	55	+	-	-
China					
Faba bean	15	2	+	-	-
Syria					
Chickpea	579	460	-	+	+
Faba bean	362	279	-	+	+
Lentil	78	46	-	+	+
Pea	21	12	-	+	+
Yemen					
Pea	35	19	-	+	+
Lebanon					
Chickpea	32	3	-	+	+
Faba bean	232	43	-	+	+
Pea	35	1	-	+	+
Lentil	13	1	-	+	+

* M-I= 1-1G5+1-3H4+1-4B12; M-II= 5-2B8+5-3D5; M-III= 5-5B8 (1, 2)

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Emerging frontiers in forest pathology

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INTRODUCTION

If one uses the first text book on the topic as starting point, the study of tree diseases is a little more than 100 years old. Thus, Robert Hartig's fundamental text published in 1874 and translated into English in 1894 as "Textbook of the diseases of trees" provided the first firm foundation for forest pathology. It is interesting, that it was not long after the publication of Hartig's forest pathology text book that chestnut blight caused by *Cryphonectria parasitica* was first found outside its native range in the United States in 1904. At the time, this might have been considered co-incidental, but it was clearly linked to growing trade, particularly between northern hemisphere countries and particularly including wood and wood products. Thus, outbreaks of devastating diseases such as white pine blister rust, Dutch elm disease and others caused by non-native pathogens first emerged more or less during the same period of time.

The study of tree diseases and the field of forest pathology have grown firmly during the course of the last Century. The causal agents of diseases of unknown aetiology have been discovered, new diseases have been recorded and the biology of the biology of many important tree pathogens has been elucidated. A suite of outstanding text books have been produced both for the teaching environment as well as for diagnosticians. Furthermore, we have celebrated the careers of many outstanding forest pathologists, working in many different parts of the world and contributing substantially to our understanding of tree pathogens and the diseases that they cause.

While forest pathology has become a well-established field of study and outstanding forest pathologists practice in many countries, there are causes for concern. In this regard, new and in some cases very serious tree diseases continue to appear regularly, both in natural forests and in plantation environments. This implies that in many situations, we are failing to manage the global threats to forests and forestry due to diseases. Ironically, there are also indications, at least in some countries that positions for forest pathologists and educators in this field are decreasing, rather than increasing in number.

NEW TREE PATHOGEN TRENDS

The threats relating to new diseases emerging from the accidental movement of pathogens from one area to another is well-recognised. This is also a category of disease that continues to increase, despite global efforts to manage the movement of plants and plant products. A worrying trend that appears to be increasing is the adaptation of pathogens to new hosts. Apparent host and vector shifts, at levels previously unexpected are occurring for host specific pathogens. Novel pathogens are also emerging through hybridisation. The processes leading to host/vector shifts and hybridisation leading to the evolution of so-called "new pathogens" are poorly understood and they deserve increased attention.

Climate change is one of the most important issues facing the world and it will clearly also impact on tree health. Very little focused research has been conducted on the likely impact of climate change on tree diseases. This is a complex area of study but it is also one that will require the attention of forest pathologists globally.

FOREST PATHOLOGY OPPORTUNITIES

New tree diseases and new categories of tree disease are emerging and this is a trend that is likely to continue to grow. This will also bring new and exciting challenges for forest pathologists. New tools and technologies continue to become available for research and these will clearly also enhance the depth and breadth of tree pathology investigations.

Identification of tree pathogens and disease diagnosis is a field that has changed markedly during the course of the last two decades. The emergence of DNA-based tools for these purposes is now commonly used and this will be increasingly true in the future. Many tree pathogens that have been treated as single species are now known to represent suites of cryptic taxa and this is already having a substantial impact on for example quarantine regulations. DNA based techniques have likewise made it possible to recognise hybrids between species and they have shifted our understanding of pathogen population biology to new and exciting levels. Furthermore, diagnostics have become much more rapid and also more accurate. These are all areas that will grow substantially in the foreseeable future.

For many years, quarantine efforts to restrict the movement of pathogens, was based on lists of names of undesirable organisms. It is now widely recognised that such lists have many flaws, many that have also emerged from the more sophisticated taxonomy and population biology tools now available. The focus has clearly shifted to understanding pathways that allow pathogens to move globally. Many opportunities remain to be explored in this domain and exciting new strategies are likely to emerge in the future

In order to understand the importance and impact of climate change on tree health, there will clearly need to be a greater focus on team research. The importance of integrating the efforts of scientists from different disciplines has long been recognised. Yet there are disappointingly few examples of tree health research conducted by formally constituted and supported multidisciplinary teams.

CONCLUSION

Forest pathology is more important today than it has ever been in the past. The threat of tree diseases in their many different manifestations has increased steadily ever since they were first formally recognised. This trend is likely to continue to grow. The challenge for forest pathologists will be to fight for a fair share of biological science budgets for tree health research. This funding can then be expended to capture the many new and exciting opportunities and technologies that can contribute substantially to improving the current and future health of the world's forests.

Variability in pathogenicity of *Quambalaria pitereka* on spotted gums

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INTRODUCTION

Quambalaria shoot blight (QSB) is a serious disease affecting the expanding eucalypt plantation estate in subtropical and tropical eastern Australia (1,2). *Quambalaria pitereka* has been isolated from foliage, stems and woody tissue of species in the genera *Corymbia*, *Blakella* and *Angophora* in Australia (1,2,3). *Quambalaria pitereka* affects the new flush of *Corymbia* foliage causing spotting, necrosis and distortion of expanding leaves and green stems.

The aim of this investigation was to identify if variation in pathogenicity levels existed between isolates of *Q. pitereka* collected from a range of species and regions in NSW and Queensland.

MATERIALS AND METHODS

Isolates of *Q. pitereka* were collected from spotted gum plantations and *Corymbia* species trials in north Queensland (NQ), south east Queensland (SEQ) and northern New South Wales (NSW). Isolates used were: Q107 from *C. torelliana* (NQ), Q147 from *C. citriodora* (NQ), Q151, Q152 from *C. variegata* (SEQ) and Q191, Q200 from *C. variegata* (NSW).

Spotted gum seedlings (1/2 sibling Woondum provenance) and cuttings were grown in steam sterilised soil mix and fertilised with slow release Osmocote[®] (Native Trees) as required and irrigated twice a day for 10 minutes each using overhead sprinklers. Glasshouse temperatures were maintained at 25–28°C during the day and 20–22°C overnight. Seedlings were grown for three months prior to inoculation with *Q. pitereka*.

Isolates of *Q. pitereka* were obtained from single lesions and grown on Potato Dextrose Agar (PDA) for 2 to 3 weeks in the dark at 25°C. A spore suspension (1×10^6 spores/ml) was obtained by washing plates with sterile distilled water (SDW) to which two drops of Tween 20 had been added prior to inoculation. Seedlings were inoculated using a fine mist spray (2.9 kPa pressure) generated by a compressor driven spray gun (Iwata Studio series 1/6 hp; Gravity spray gun RG3, Portland, USA), to the upper and lower leaf surfaces of the seedlings until runoff was achieved. All seedling trees were covered with plastic bags immediately after inoculation to maintain high humidity levels and to increase the period of leaf wetness. Bags were removed after 48 hours and plants watered using overhead irrigation systems twice a day for a period of 10 minutes. Sub-samples of the spore suspension applied to the trees were placed onto PDA and incubated at 25°C for 48 hours to ensure that the spores were viable.

Each treatment was replicated six times with a single inoculation per tree and assessed for disease incidence and severity 14 days later. A QSB score was then calculated to compare levels of pathogenicity between isolates. A comparison between isolates was done using ANOVA (StatView[®]).

RESULTS

Significant variability in pathogenicity was observed between isolates. Isolate Q152 was significantly more virulent than all other isolates.

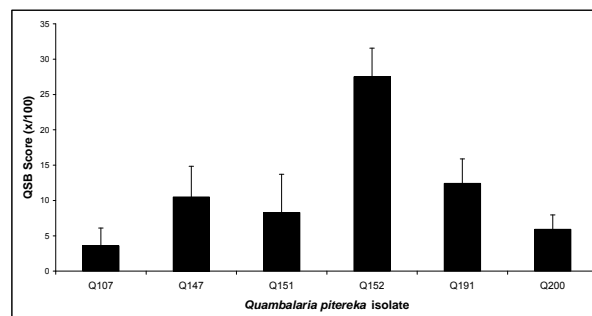


Figure 1. Comparison of pathogenicity of a range of isolates of *Q. pitereka* on spotted gum, Woondum provenance.

DISCUSSION

Variability in pathogenicity between isolates of *Q. pitereka* has not previously been studied. This preliminary study using isolates from a range of regions identifies only a small degree of variability. More extensive studies are needed to investigate the significance of pathogen virulence on disease development, especially in relation to selecting for disease resistance. Further studies are required to investigate *Q. pitereka* population genetics and potential variability in isolate virulence.

ACKNOWLEDGEMENTS

We thank Queensland Primary Industries Innovation and Biosecurity Program Investment, Forest Plantations Queensland, Integrated Tree Cropping, Forest Enterprises Australia and Forests NSW for providing the necessary funding for this research.

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Movement of pathogens between horticultural crops and endemic trees in the Kimberleys

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INTRODUCTION

Recently a survey of endophytes associated with baobabs (*Adansonia gregorii*) and associated tree species in the Kimberleys, Western Australia has resulted in the description of seven new species in the Botryosphaeriaceae (Pavlic *et al.* 2008). Additionally several common species of *Lasiodiplodia*, (*L. theobromae*, *L. pseudotheobromae* and *L. parva*) were also isolated as endophytes of endemic tree species.

Concurrently, surveys in the Ord River Irrigation Area (ORIA) have revealed *Mangiferum indica* trees showing symptoms of dieback and cankers. In this project we isolated, identified and determined the pathogenicity of fungi associated with these cankers.

MATERIALS AND METHODS

Isolation and identification. Fungi were isolated from cankers using standard techniques. Due to the similarity in morphological features among the Botryosphaeriaceae, molecular identification was conducted by extracting DNA, amplifying and sequencing the ITS and EF1- α gene regions (Burgess *et al.* 2005) and conducting phylogenetic analysis to identify cryptic species.

Pathogenicity trials. Trials were conducted using unripe but mature Kensington pride mangoes. The mangoes were washed in water then submerged in 1.5% NaOH (Bleach) solution for 2 minutes to disinfect. Mangoes were then allowed to air dry and were stored overnight at temp 28–33°C. Mangoes were inoculated with 11 fungal isolates (9 replicates per isolate), by wounding with the tip of a sterile pipette and immediately placing a colonised agar plug mycelia side down over the wound. After 6 days the lesions were measured.

RESULTS AND DISCUSSION

Seven species from the Botryosphaeriaceae were isolated from mangoes in the ORIA. Three of these species, *L. theobromae*, *N. ribis* and *N. dimidiatum* are known pathogens of mangoes causing either cankers or post-harvest disease. Four species, *P. adansoniae*, *N. novaehollandia*, *L. pseudotheobromae* and *L. parva* have not been reported previously from mangoes, but are commonly found as tree endophytes in the surrounding region.

All tested species were pathogenic to mangoes, with *Lasiodiplodia theobromae* causing the largest lesion followed by the *Neoscytalidium spp.* *P. adansoniae* caused the smallest lesions.

Table 1. Species of Botryosphaeriaceae isolated as endophytes of endemic trees in the Kimberleys and causing disease in mangoes in Kununurra

Species	Mangoes	Trees
<i>Pseudofusicoccum adansoniae</i>		#
<i>Pseudofusicoccum kimberleyense</i>		#
<i>Pseudofusicoccum ardesiacum</i>		#
<i>Lasiodiplodia theobromae</i>		
<i>Lasiodiplodia pseudotheobromae</i>		
<i>Lasiodiplodia parva</i>		
<i>Lasiodiplodia margaritacea</i>		#
<i>Lasiodiplodia crassispora</i>		
<i>Dothiorella longicollis</i>		#
<i>Fusicoccum ramosum</i>		#
<i>Neoscytalidium novaehollandia</i>		#
<i>Neoscytalidium dimidiatum</i>		
<i>Neofusicoccum ribis</i>		

denotes species newly described by Pavlic *et al.* 2008

Common endophytes and latent pathogens of mangoes, *Neofusicoccum mangiferae*, *N. parvum* and *Botryosphaeria dothidea*, were not isolated in the ORIA. In addition *N. ribis* was isolated very rarely. The most commonly isolated pathogens were *L. theobromae*, *P. adansoniae* and the two *Neoscytalidium* spp. interestingly these fungi are also common in the surrounding endemic vegetation. It appears that many of the pathogens of mangoes in the ORIA have moved onto the trees from the surrounding environment rather than arriving with nursery stock. The differences in the species found in the ORIA compared with other mango growing regions could be due to the geographic isolation of the region.

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Pathogenicity of *Phytophthora multivora* to *Eucalyptus gomphocephala* and *E. marginata*

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INTRODUCTION

Since the early 1990s there has been a significant decline of *E. gomphocephala*, and more recently *E. marginata*, in the tuart forest in tuart woodland in Yalgorup National Park SW Western Australia, although no satisfactory aetiology has been established to explain the decline. Characteristics of the canopy dieback and decline distribution are reminiscent of other forest declines known to involve *Phytophthora* soil pathogens and indicate that a *Phytophthora* species may be involved in the decline. In 2007 isolates of *Phytophthora multivora*, recently described by (1), were recovered from rhizosphere soil of declining or dead trees of *Eucalyptus gomphocephala* and *E. marginata*. For *E. gomphocephala* and *E. marginata*, the pathogenicity of *P. multivora* was tested: *ex situ* on seedlings using a soil infestation method; and *in situ* on stems using an under bark infestation method.

MATERIALS AND METHODS

Ex situ soil inoculation trial: The roots of *E. gomphocephala* seedlings, grown in neutral coarse river sand, were infested with a vegetable juice—vermiculite medium colonised with five isolates of *P. multivora* isolated across the Yalgorup decline and two isolates of *P. cinnamomi*; while the roots of *E. marginata* seedlings were infested with one isolate of *P. multivora* as described (2). After one year the roots of infested seedlings were scanned and the lengths of roots of different diameters were calculated using the WINRHIZO Pro V 2007d software (Reagent Instruments, Québec, Canada). Isolates were recovered from the roots of all infested seedlings.

In situ under bark inoculation trial: The stems of less than 1.5 m tall *E. gomphocephala* saplings, naturally growing on site in Yalgorup National Park, were under bark inoculated with five isolates of *P. multivora*; while saplings of *E. marginata* were inoculated with one isolate of *P. multivora* as described (3). After nine weeks saplings were harvested and lesion lengths calculated.

RESULTS

Ex situ soil inoculation trial: Preliminary results from the *ex situ* soil infestation trial indicate that *E. gomphocephala* seedlings treated with *P. multivora* isolate Pm1 and Pm2 and *P. cinnamomi* isolates Pc1 and Pc2, had significantly less roots between 0–2 mm in diameter compared to the control (Figure 1). *Eucalyptus gomphocephala* seedlings infested with *P. multivora* isolates Pm3, Pm4 and Pm5 did not have significantly less roots compared to the control across any size class. *Eucalyptus marginata* seedlings infested with *P. multivora* isolate Pm1, did not have significantly less roots compared to the control across any size class.

In situ under bark inoculation trial: Saplings of *E. gomphocephala* and *E. marginata* inoculated with all *P. multivora* isolates had significantly longer lesions compared to the control. When harvested the average lesion length on *P. multivora* inoculated *E. gomphocephala* and *E. marginata* seedlings was 13.6 mm and 90.5 mm respectively.

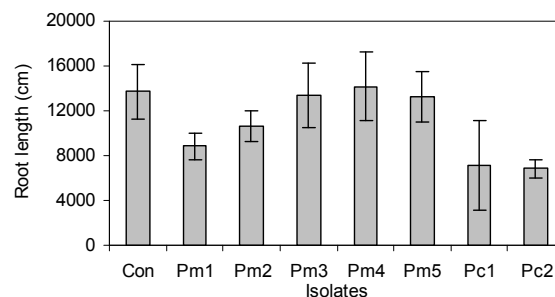


Figure 1. Length of live roots (mean ± SE) of *E. gomphocephala* seedlings for roots 0–2 mm in diameters Con, control; Pm 1–5, *P. multivora* isolates 1–5; Pc 1–2 *P. cinnamomi* isolates.

DISCUSSION

The significant reduction in root diameter in *E. gomphocephala* seedlings after infestation with isolates Pm1 and Pm1 indicates that *P. multivora* is a pathogen of *E. gomphocephala* under glasshouse conditions and may be a significant soil pathogen to *E. gomphocephala* in the field. The variation in pathogenicity of *P. multivora* isolates used in the soil infestation trial suggests that there is variation in the pathogenicity of *P. multivora* isolates within the field.

The significant lesion lengths measured in *E. gomphocephala* and *E. marginata* sapling inoculated with *P. multivora* isolates confirms that *P. multivora* is a pathogen to both host species under conditions where *P. multivora* can colonise the vascular tissue in the field. The lesion lengths indicate that *P. multivora* can be especially *E. marginata* saplings.

The variation in pathogenicity observed between isolates and species in both soil infestation and under bark inoculation trials suggests that *P. multivora* can be significantly aggressive to both *E. gomphocephala* and *E. marginata*; although further research is needed to understand the population dynamics of the pathogen and its impact within the tuart decline in Yalgorup National Park.

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Microscopy of progressive decay of fungi isolated from meranti tree canker

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INTRODUCTION

White rot basidiomycetes are especially important in the forest ecosystem because they are the only fungi capable of degrading all cell wall components (cellulose, lignin, hemicelluloses) of wood. Micromorphological aspects of two main types of white rot, selective delignification and simultaneous rot, have been distinguished [1]. Light red meranti (*Shorea smithiana*) and yellow meranti (*Shorea gibbosa*) trees which growing in one area of natural dipterocarps in Kalimantan, Indonesia inhabited by white-rot fungi, *Schizophyllum commune* [2] and *Phlebia brevispora* [3], respectively. It is of interest that *S. commune* and *P. brevispora* in the decayed wood of standing *S. smithiana* and *S. gibbosa* and have been causing simultaneous decay.

This study was performed to confirm their progressive decay patterns in artificial laboratory conditions (*in vitro*).

MATERIALS AND METHODS

Fungal strain and decay test procedure. The decay fungi isolated from decayed wood of *S. smithiana* and *S. gibbosa* cankerous trees, were genetically identified by their ITS sequence as *Schizophyllum commune* [2] and *Phlebia brevispora* [3], respectively. Twelve sound wood-blocks (20 x 20 mm in cross-section x 10 mm in length) obtained from the uninfected portions of the stem disks were inoculated with the identified fungi, and incubated in accordance with the JIS K 1571 soil-block test procedure [4].

Microscopic observations. Various stages of the decay wood were examined using light and scanning electron microscopy. Six exposure times were analyzed: 2, 4, 6, 8, 10 and 12 weeks.

RESULTS

After 12 weeks of exposure in the laboratory decay test, wood blocks of *S. smithiana* that had been inoculated with *S. commune* fungus sustained an average weight loss of 1.82%, whereas *P. brevispora* decayed *S. gibbosa* wood more aggressively than *S. commune* (Table 1).

Table 1. Weight loss in *S. smithiana* and *S. gibbosa* wood infected with *S. commune* and *P. brevispora*, respectively.

Incubation period (weeks)	Weight loss percentage (Mean±SE)	
	<i>S. smithiana</i>	<i>S. gibbosa</i>
2	0.42 ± 0.32	0.91 ± 0.10
4	0.50 ± 0.44	2.24 ± 0.60
6	0.54 ± 0.50	5.02 ± 1.03
8	0.60 ± 0.62	8.23 ± 1.22
10	0.80 ± 0.40	11.80 ± 5.15
12	1.82 ± 0.40	12.34 ± 2.76

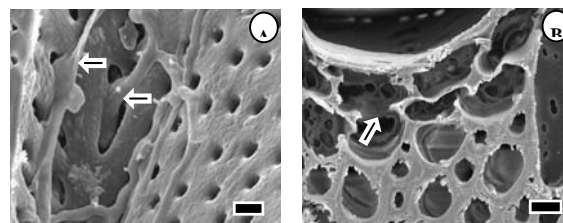


Figure 1. Decay wood caused by fungus for 12 weeks incubation. (A) Hyphal branches of *S. commune* fungus passed through pits in vessels of *S. smithiana* (arrows). Bar 5 μm; (B) Erosion channels in parenchyma cells adjacent to infected vessels of *S. gibbosa* (arrow). Bar 10 μm.

DISCUSSION

Slight erosion of wood cell walls in *S. smithiana* over 12 weeks' incubation was classified as the early stage of simultaneous decay, and showed a similar pattern to that observed in naturally decayed wood samples.

P. brevispora reduced *S. gibbosa* wood weight by 0.91–12.34% and produced progressive simultaneous decay over 2–12 weeks' incubation *in vitro*. The first 6 weeks of incubation was classified as the early stages decay, in which pit erosion and slight erosion of cell walls facilitated hyphal between cells. Numerous and conspicuous holes as well as erosion troughs in cell walls, which were found at the end of 8 weeks' incubation, showed that an intermediate stage of decay had occurred. Furthermore, complete degradation of wood cell components, termed the advanced stage of decay, was found in some areas of wood blocks after 12 week's incubation.

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Optimising conditions to investigate gene expression in pathogenic *Streptomyces* using RT-qPCR

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INTRODUCTION

Pathogenic *Streptomyces* species cause common scab of potato. The pathogen produces a phytotoxin, thaxtomin A, that induces a host response that creates the corky symptoms of common scab that can be superficial, raised, netted or deep pitted. QPCR is being increasingly used as a method for mRNA quantification but only a relatively few studies have reported the use of RT-qPCR for plant pathogens. This study aimed at optimising total RNA isolation from pathogenic *Streptomyces* spp. and using RT-qPCR conditions to quantify thaxtomin A transcripts.

MATERIALS AND METHODS

Pathogenic *Streptomyces* strain. *Streptomyces scabies* strain S29 was grown on yeast malt extract agar (YME) at 27°C. After one week S29 was inoculated into 3 types of growth media (yeast malt extract broth YME, thaxtomin defined medium broth TDM, oatmeal broth OM) both with and without cellobiose (1). The inoculated media were incubated at 27°C on a rotating shaker for one week then filtered through cheese cloth to separate the *Streptomyces* cultures from the media. The cultures were used fresh or stored at -20°C until ready to extract RNA.

RNA extraction. RNAqueous 4 PCR (Ambion) for isolation of DNA-free RNA was used to extract total RNA from the *Streptomyces* cultures according to the manufacturers instructions.

cDNA synthesis and quantification. Total RNA was treated with DNaseI and ABI High capacity cDNA reverse transcription kit was used to transcribe RNA into cDNA according to the manufacturers instructions. The resulting cDNA was quantified using Nanodrop ND-1000 Spectrophotometer.

cDNA dilutions and qPCR. The cDNA was diluted to the following concentrations 500, 250, 125 and 62.5 ng/5 µL and replicated 3 times. QPCR was performed in 0.2 mL tubes in a Rotor Gene 3000 machine (Corbett Research). Each 25 µL reaction consisted of 50 ng template cDNA, 1x SYBR qPCR Super Mix (Invitrogen) 0.1 µM of primers TxTATq1 and TxTATq2 (2). The thermal cycle protocol was 50°C for 2 min, 95°C for 2 min and 45 cycles of 95°C for 15 sec and 60°C for 60 sec and Melt cycle of 60–95°C in 1°C intervals. Negative controls without cDNA template were run with every assay to rule out contaminations.

RESULTS

The RNA isolation method RNAqueous 4 PCR provided a high quantity of high integrity RNA. The RNA was transcribed into cDNA using the ABI High capacity cDNA reverse transcription kit and gave good quality cDNA. The transcript levels of thaxtomin A were quantified using SYBR qPCR with primers specific to thaxtomin A. A standard curve was constructed from the means of the serially diluted cDNA replicates (Figure 1). The small standard errors with these replicates demonstrated that the assay is reproducible and highly robust. The transcript levels of thaxtomin A were greater in media amended with cellobiose than without, with oatmeal broth having the highest transcript levels as expressed by Ct value (Figure 2).

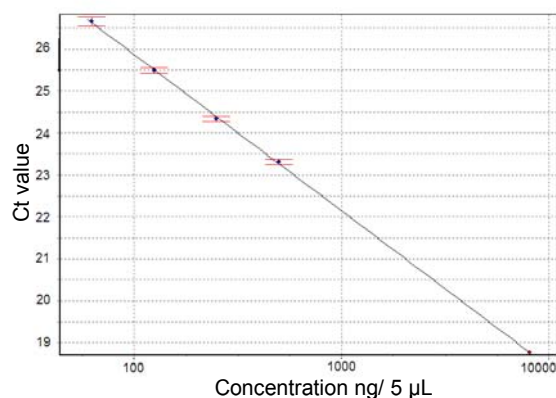


Figure 2. Standard curve obtained from serially diluted cDNA. Ct values are the means of 3 replicates. Error bars represent standard error of the means.

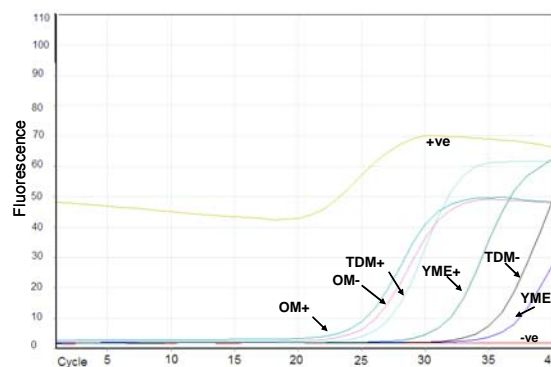


Figure 3. Amplification curves for amended media. OM oatmeal, OM+ oatmeal with cellobiose, TDM thaxtomin defined medium, TDM+ thaxtomin defined medium with cellobiose, YME yeast malt extract, YME+ yeast malt extract with cellobiose.

DISCUSSION

This proof of concept study optimised the RNA extraction method from pathogenic *Streptomyces* and RT-qPCR conditions to quantify thaxtomin A transcripts. The levels of thaxtomin A varied depending on the growth media used, with oatmeal plus cellobiose giving the highest quantity. The next stage of this research will be to use these optimum conditions to study gene expression of pathogenic *Streptomyces* spp.

ACKNOWLEDGEMENTS

This project was facilitated by HAL in partnership with the processing potato industry, funded by the processing potato levy and voluntary contributions from industry partners. The Australian Government provided matched funding for HAL's R&D activities. DPI Victoria contributed funding to this project.

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Fusarium oxysporum and *Pythium* species associated with vascular wilt and root rots of greenhouse cucumbers

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INTRODUCTION

Fusarium wilt of cucumber caused by *F. oxysporum* f.sp. *cucumerinum* (F.o.cuc) and root rots caused by *Pythium* species have a worldwide distribution. *F. oxysporum* f. sp. *radicis-cucumerinum* (F.o.r-cuc) has more recently been described in Europe and North America causing stem and root rots of greenhouse cucumbers (1, 2). F.o.r-cuc was shown to be genetically distinct from F.o.cuc (3).

Pythium species are commonly associated with roots from a wide botanical host range (4). Member species and isolates can be aggressive pathogens, weakly pathogenic, secondary invaders, saprophytes or hyperparasites.

Except for the one previous study of *Fusarium* wilt in Australian greenhouse cucumbers (5), the causes and severity of wilt, stem and root rots are unknown.

Preliminary surveys of greenhouse cucumbers in crops grown in the peri-urban districts of Sydney revealed severe vascular wilt, stem and root rots associated with heavy crop losses. The purpose of this study was to identify potential agents responsible for these diseases and to determine their significance across major Australian production areas.

MATERIALS AND METHODS

Greenhouse cucumber crops were surveyed across major Australian production areas in NSW, Qld., S.A. and W.A. between 2001 and 2006. Cucumber plants were assessed *in-situ* for stunting, wilting, discolouration of hypocotyl tissue, and pink-orange sporulation on stem lesions. Samples of diseased plants that were not permanently wilted were collected, transported to a laboratory and processed within 24 hours where possible. Growers freighted further samples directly to the laboratory.

Sub-samples of symptomatic roots and stems were washed clean of soil or media, surface-sterilised in a hypochlorite solution and rinsed in sterile-distilled water.

Pythium species were isolated from roots and crown tissue that had been plated to potato carrot agar amended with 5 ppm pimarinic acid and 10 ppm rifampicin. *Fusarium* species were similarly isolated from roots and stem vascular tissue plated to ¼-strength potato dextrose agar amended with 100 ppm novobiocin. Plates were incubated at 25°C and examined for the presence of typical features of these genera under a compound light microscope. *Pythium* species were sub-cultured and identified (4). *Fusarium* isolates were single-spored and identified (6).

Representative isolates of *Pythium* taxa were further characterised by sequence analysis of ITS rDNA regions and compared with published sequences (7).

Fusarium isolates were compared with F.o.cuc and F.o.r-cuc reference isolates from overseas by determining their vegetative compatibility groupings (VCGs), repPCR profiles and sequences of their β-tubulin, calmodulin and α-elongation factor genes.

RESULTS AND DISCUSSION

Pythium isolates were assigned to nine taxonomic groups. Seven conformed to named species: *P. aphanidermatum* (Edson) Fitzpatrick, *P. coloratum* Vaartaja, *P. irregulare* Buisman, *P. mamillatum* Meurs, *P. oligandrum* Drechsler, *P. spinosum* Sawada, and *P. ultimum* Trow var. *ultimum* Trow. The remaining two taxa were placed in *Pythium* 'Group T' and the *Pythium* species 'HS Group' (4).

One unique VCG of *F. oxysporum* dominated in all production areas. It also had a unique repPCR profile while gene sequences aligned closely with F.o.cuc. A number of other isolates each had unique VCGs while they shared similar repPCR profiles. A subset of this group clustered with gene sequences of F.o.r-cuc. Further VCG studies will be required for F.o.cuc isolates since the publication of four new groups from China (8).

This study confirmed that F.o.cuc is the major subspecies of *Fusarium oxysporum* associated with greenhouse cucumber vascular wilt disease in Australia. Isolates consistent with F.o.r-cuc were also identified as a minor population. Pathogenicity and host range studies completed the characterisation of representative *Fusarium* and *Pythium* isolates. Results of these studies will be reported elsewhere.

ACKNOWLEDGEMENTS

NSW DPI, the Australian Government and HAL Ltd funded this study. L. Gunn, S. Azzopardi, F. Lidbetter, C. Howard and S. Peterson assisted with molecular studies. Thanks also to B. Summerell and E. Liew for guidance and use of the laboratory at the RBG, Sydney.

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Fusarium oxysporum f. sp. *fragariae*: a main component of strawberry crown and root rots in Western Australia

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INTRODUCTION

Root and crown rots are important diseases of strawberry crops worldwide. The fungi *Phytophthora* spp., *Verticillium* spp., *Fusarium* spp., *Gnomonia fragariae* and *Colletotrichum* spp. have been reported as causal agents of strawberry crown and root rot, and they caused considerable yield reduction. In many cases the causes of root rot are referred to several pathogens of which *Pythium* spp., *Rhizoctonia* spp., *Cylindrocarpon* spp., *Phoma* spp., *Coniothyrium* spp. and *Fusarium* spp. have been the most common in the root rot complex (1, 3). *Fusarium oxysporum* f. sp. *fragariae* has been reported in Queensland, Western Australia and Japan as an important pathogen of strawberry (2, 3, 4).

MATERIALS AND METHODS

Field survey. During the surveys conducted a high incidence of strawberry death was observed in coastal districts up to 50 km north of Perth areas in 2005 and 2006. A total of 50 partially diseased and asymptomatic plants were randomly collected from ten major strawberry fields. Roots were carefully washed under running tap water and the crown of each plant was dissected lengthwise. Vascular discolouration of the crown and root was evident on some of the samples collected.

Isolation method. Crowns and roots of diseased and asymptomatic plants were surface-sterilised by immersion in a 1.25% aqueous solution of sodium hypochlorite for 1 min, rinsed in sterile water and dried in a laminar flow cabinet. Out of 50 samples 500 root and crown specimens with equal numbers of 10 pieces per sample were selected randomly. Specimens were separately placed on potato dextrose agar (PDA), water agar and selective media (P10VPH and P10VP) and then incubated at 22 ± 3°C. Emerged fungal colonies were sub-cultured on carnation leaf agar, PDA and V-8 juice agar and incubated at 25°C with a 12 h dark and light cycle. Growth rate, colony morphology and morphological characteristics of the isolated fungi were determined.

Pathogenicity test. The pathogenicity of 10 *Fusarium* isolates was tested on *Fragaria × ananassa* cv. Camarosa, *Lycopersicon lycopersicum* cv. Petula and *Cucumis sativus* (Lebanese cucumber) in a glasshouse experiment. Strawberry runners and 4-week-old seedlings of tomato and cucumber were inoculated by dipping the roots in a spore suspension (10⁵ spores/mL) before planting. Controls were dipped in tap water.

RESULTS AND DISCUSSION

During the surveys, a high incidence of decline and death of strawberries was observed. Mortality of Camarosa and Gaviota varieties of strawberry (*Fragaria × ananassa*) was between 0 and 60% in some strawberry fields.

Of the 500 root and crown specimens, *Fusarium* spp. was consistently isolated from diseased plants. *Fusarium* isolates used were pathogenic on the strawberry runners but non-pathogenic on the tomato and cucumber plants tested. On the basis of morphological characteristics and pathogenicity tests, *F. oxysporum* f. sp. *fragariae* was identified as a main component of root and crown rots of strawberries. Average percentage

recovery of the fungi (Table 1) was indicated that *Fusarium* spp. (74%) was predominant while *Phytophthora* spp. (21%), *Pythium* spp. (23.5%), *Phoma* spp. (3%), *Rhizoctonia* spp. (9%), *Colletotrichum* spp. (1.5%) and *Macrophomina* spp. (16%) were minor components of root and crown rots of strawberries. In most cases combination of fungi were recovered from both root and crown strawberry samples tested. A culture of *F. oxysporum* f. sp. *fragariae* has been deposited in the culture collection of Department of Agriculture and Food Western Australia as WAC 12708.

Table 1. Average percentage of fungi associated with root and crown rots of Camarosa cultivar in 2005 and 2006

Recovered Fungi	2005 and 2006 Total recovery %	
	Diseased	Healthy*
<i>Fusarium oxysporum</i> f. sp. <i>fragariae</i>	74.0	10.0
<i>Phytophthora</i> spp.	21.0	1.5
<i>Pythium</i> spp.	23.5	9.0
<i>Phoma</i> spp.	3.3	0.0
<i>Rhizoctonia</i> spp.	9.3	2.0
<i>Colletotrichum</i> spp.	1.5	0.0
<i>Macrophomina</i> spp.	16.0	1.0
Others	9.0	1.0

* Asymptomatic plant

Strawberry root and crown rots seem to be caused by diverse fungi. Determining the specific fungus or fungi causing crown and root rots is complicated, because the fungi isolated from diseased tissue may only be capable of causing disease under specific conditions or only in specific associations with other organisms.

ACKNOWLEDGEMENT

The authors would like to thank the Western Australia strawberry industry for financial support.

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Evaluation of resistant rootstocks for control of *Fusarium* wilt of watermelon in Nghe An Province, Vietnam

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INTRODUCTION

Fusarium wilt of watermelon, caused by *Fusarium oxysporum* f.sp. *niveum*, has caused serious losses in watermelon crops in Nghia Dan District, Nghe An Province, Vietnam over the past two years, and is threatening the viability of the industry (1). We demonstrated that the local watermelon cultivar could be grafted successfully onto a resistant rootstock in 2008. In this paper we report on the evaluation of a range of potential resistant rootstocks in relation to graft compatibility and watermelon production and quality, and the assessment of three rootstock-scion combinations under commercial conditions.

MATERIALS, METHODS AND RESULTS

We evaluated the local cultivar Bau trang (*Lagenaria sinceraria*) together with five hybrid cucurbits for use as resistant rootstocks. The hybrids, provided by Syngenta, Thailand, were Kazako, Carnivar and Bulrojangsaeng (*S. sinceraria*), and Emphasis and Argentaria (pumpkin). The simple grafting technique as refined by Syngenta, is efficient and can be taught quickly to farmers. The three basic tools used by our cooperating farmers are a razor blade, and home-made scalpel and stylet (Figure 1).



Figure 1. Basic tools used for grafting watermelon onto *Fusarium* wilt resistant rootstock. Home made scalpel (top), stylet (middle) and razor blade (bottom)

The grafting process involves excising the stem of the rootstock seedling immediately above the cotyledons, at the first true leaf stage. A narrow cone shaped slot is then made in the stem with the stylet. The upper part of the stem of a watermelon seedling (0.8 to 1.0cm long), at the cotyledon stage, is then removed with a razor blade, making an oblique transfer cut. The scion is then inserted in the slot in the rootstock stem. The grafting process takes about 10 to 15 seconds. The success rate is approximately 80% or greater depending on experience. A successful graft is shown in Figure 2.



Figure 2. Successful graft of watermelon on resistant rootstock (A) and graft junction on mature plant (B).

The Bulrojangsaeng hybrid was the best rootstock on all criteria but the farmers consider it too expensive. Consequently the Bau trang cultivar was used as a resistant rootstock in an initial 0.1ha field trial in soil with a history of severe watermelon wilt, in spring 2009. Two watermelon cultivars from Syngenta, Thuy Loi and Phu Dong, were used as scions. The grafted plants were not affected by *Fusarium* wilt and produced marketable fruit.

Staff from Nghe An Plant Protection Sub-Department and Syngenta held farmer field schools on *Fusarium* wilt and the grafting technique in March, 2009. A cooperating farmer planted an eight-hectare summer crop of grafted seedlings in late April, 2009. He used the Bau trang rootstock and two watermelon cultivars in his trial, Hac My Nhan and Dat Viet (Nong Viet Company), selections based on cost of seed. The results of this planting will be reported.

DISCUSSION

The use of watermelon grafted onto resistant rootstocks has been shown to be a successful approach for the prevention of *Fusarium* wilt in soils with high inoculum levels. The cost of seed in relation to yield, fruit quality and market acceptance in Hanoi will determine the preferred combinations of scions and rootstocks. The widespread use of tomatoes grafted onto resistant rootstocks for control of bacterial wilt in Vietnam (2) indicates the potential for using resistant rootstocks to prevent losses from a range of diseases caused by soil-borne pathogens.

ACKNOWLEDGEMENTS

Financial support from the Australian Centre for International Agricultural Research (ACIAR CP/2002/115) is gratefully acknowledged.

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Bunch rot diseases and their management

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INTRODUCTION

Bunch rot diseases are of concern in all regions of the world where grapes are grown but nowhere are they more problematic than in moist and temperate growing regions. Among the most important bunch rot diseases in the southeastern United States are black rot, phomopsis, botrytis, bitter rot, ripe rot, and sour rot. This paper summarises a series of studies designed to gain a better understanding of the biology and epidemiology of the pathogens that cause bitter rot [*Greeneria uvicola* (Berk. & Curtis) Punith.] and ripe rot [homothallic and heterothallic strains of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (teleomorph *Glomerella cingulata* (Stonem.) Spauld. & Schrenk) and *C. acutatum* J.H. Simmonds (teleomorph *G. acutata* Guerber & Correll)] and how to manage them.

BIOLOGY AND EPIDEMIOLOGY OF *G. UVICOLA* AND *COLLETOTRICHUM* SPP.

Fruit susceptibility to *G. uvicola* and *Colletotrichum* spp. through the growing season. From 2003–2007 a series of studies were conducted to determine the period during the growing season that fruit were susceptible to infection by *G. uvicola* and homothallic isolates of *C. gloeosporioides*. Fruit of Chardonnay, Merlot, and Cabernet Franc were susceptible to infection by *G. uvicola* from bloom until harvest but were most susceptible just prior to and during véraison. Although fruit were susceptible to *C. gloeosporioides* from bloom to harvest, the period of peak susceptibility was not as obvious. Chardonnay was most susceptible at bloom and véraison, Seyval blanc during bloom, post-bloom, and véraison, and Cabernet Franc at post-bloom, closing, véraison, and preharvest.

Influence of temperature and leaf wetness on infection of fruit by *G. uvicola*. Detached fruit of *V. vinifera* (cv Chardonnay, Cabernet Franc, and Cabernet Sauvignon) were atomised with a conidial suspension of *G. uvicola* (10^5 spores/ml), placed in moist chambers and subjected to 14, 18, 22, 26, and 30°C for 6, 12, 18, or 24 h of wetting. Optimum conditions for infection were 6 or 12 h of wetting and temperatures ranging from 22.4 to 24.6°C (mean=23.3°C) (1).

Relative susceptibility of cultivars to *G. uvicola* and *Colletotrichum* spp. Fruit of 38 grape cultivars or selections were evaluated for their susceptibility to *G. uvicola* while 35 cultivars or selections were tested for their susceptibility to a homothallic isolate of *C. gloeosporioides* in a laboratory assay

There was a wide variation in the susceptibility of fruit to *G. uvicola*. Fruit of *V. vinifera* were more susceptible than the French American hybrids. *V. aestivalis* Cynthiana was the most resistant to *G. uvicola* (1). There was also a wide range in the susceptibility of cultivars and selections to *C. gloeosporioides*; however, there was not a clear difference between the relative susceptibility of *V. vinifera* and hybrids to *C. gloeosporioides*. Early harvest Cynthiana, Chardonnay, Merlot, Petit Syrah, and Pride were among the most susceptible cultivars to *C. gloeosporioides*.

Population structure of *Colletotrichum* spp. associated with ripe rot of grapes. This study was conducted from 2004–2007 with the objectives of determining the population structure of

Colletotrichum species in the diverse geographical regions of North Carolina and to examining differences based on host genera. The relative proportion of *Colletotrichum* spp. in a vineyard varied with the cultivar/species present and location (2).

MANAGEMENT

Effect of cane pruning. An experiment was conducted from 2004–2006 on the cultivars Chardonnay, Merlot, and Cabernet Sauvignon in a 12-year-old vineyard in the Piedmont of North Carolina designed to evaluate the effect of cane pruning vs spur pruning on the incidence and severity of bitter rot and ripe rot. Cordons 1, 2, and 3-years-old were established during the course of the experiment and the incidence and severity of bitter rot, ripe rot and sour rot was compared to the 12-year-old spur pruned vines. There was a significant reduction in the incidence and severity of bitter rot and ripe rot in the first year which carried over during the 3 years of the study. Cane pruning did not reduce the incidence of sour rot, but did reduce its severity.

The disease management program for ripe rot and bitter rot in the southeastern US. The backbone of the disease management program in the southeastern US is cultural practices which are designed to reduce the initial inoculum and create an environment within the vine canopy less favorable for disease development. However, a fungicide program beginning at bloom and continuing until harvest is necessary to effectively manage summer bunch rot diseases. Captan, applied on a 10–14 day interval is the principle fungicide used from closing to harvest.

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Inoculum and climatic factors driving epidemics of *Botrytis cinerea* in New Zealand and Australian vineyards

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INTRODUCTION

Botrytis bunch rot (botrytis) reduces grape yield and wine quality in seasons when wet weather occurs during grape ripening. Grape growers need to be able to predict when there is a high risk of botrytis so that fungicide applications, vine canopy management and harvesting operations can be more effectively planned. This study investigated climatic predictors of harvest botrytis severity that were measured in non-fungicide treated plots between key vine growth stages: 1) early season (flowering to pre-bunch closure (PBC)), 2) mid season (PBC to beginning of ripening (veraison)), 3) late season (veraison to harvest).

MATERIALS AND METHODS

Weather data came from weather stations within 1–5 km of each vineyard trial site (1). Relationships between harvest botrytis severity (2) and rainfall, daily mean, minimum and maximum temperature and surface wetness duration were investigated. Surface wetness duration and temperature during wetness were summarised using the “Bacchus” model (3) from Hortplus™ (www.hortplus.com).

RESULTS AND DISCUSSION

There were strong regional associations between harvest botrytis severity and some climatic variables during some growth stage intervals (Figures 1–3). The figures show the 3% severity threshold at which wineries may impose price penalties for botrytis-affected grapes. Amount of rainfall, even late in the growing season, was, surprisingly, a poor predictor of harvest botrytis severity. Further research is using the relationships found in this study, together with fungicide and vine management factors, to develop a botrytis risk prediction model.

ACKNOWLEDGEMENTS

This study was funded by New Zealand Winegrowers, the Australian Grape and Wine Research and Development Corporation, the N.Z. Foundation for Research, Science and Technology (C06X0810) and Plant and Food Research.

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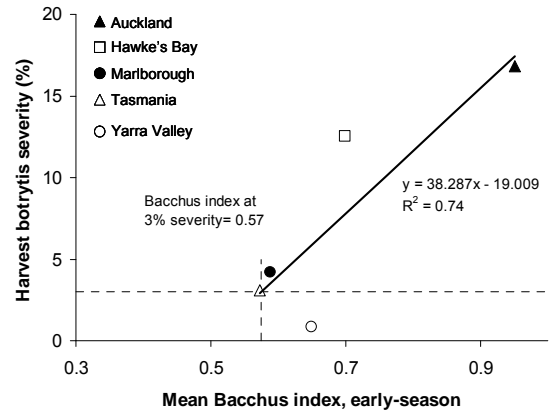


Figure 1. “Bacchus” index (surface wetness duration and temperature) was the most useful early-season indicator of harvest botrytis severity.

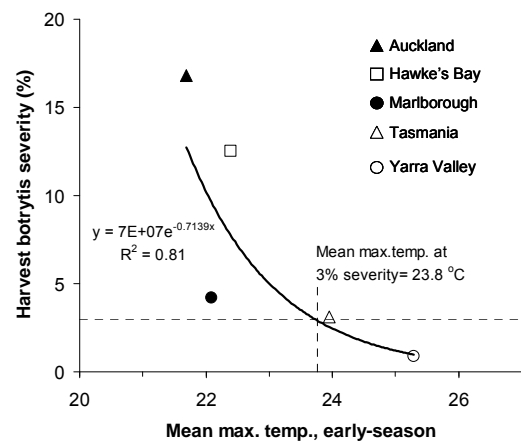


Figure 2. Mean daily maximum air temperature in the early part of the season was strongly inversely related to harvest botrytis severity.

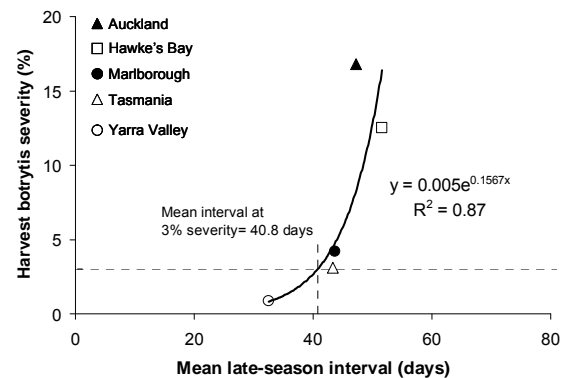


Figure 3. A longer ripening period (late-season interval) was associated with greater harvest botrytis severity. This regional trend is often reflected in individual vineyards, where a delay in harvest date to achieve higher grape sugar content is accompanied by increased risk of botrytis.

Infection of apples by *Colletotrichum acutatum* in New Zealand is limited by temperature

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INTRODUCTION

Colletotrichum acutatum infects the surface of apples in New Zealand to cause small, 1–2 mm diameter dark spots, usually on the side of the fruit exposed to the sun, which can enlarge to cover the entire fruit surface with one or several orange, sporulating lesions. Infection eventually results in fruit drop. Symptoms express in summer. Little is known of the epidemiology of *C. acutatum* infecting apples, either in New Zealand or elsewhere (1). A study was conducted to investigate the epidemiology of this fungus on apples in New Zealand, to facilitate the design of strategies to achieve control with no residues.

MATERIALS AND METHODS

Laboratory inoculations. ‘Royal Gala’ apples were harvested monthly starting immediately after fruit set in November 2005 until harvest in 2006. At each time, apples were wound-inoculated with 10^6 spores/ml *C. acutatum* and placed at 5, 10, 15, 17.5, 20, 25 or 30°C in humid conditions for 7 days. Lesion diameter was then measured.

Field inoculations. ‘Royal Gala’ apples in two orchards in each of three major apple growing regions in New Zealand, viz. Waikato, Hawke’s Bay and Nelson, were wound-inoculated monthly with 10^6 spores/ml *C. acutatum* beginning in November 2005 until harvest in February 2006. Lesion diameter was then measured.

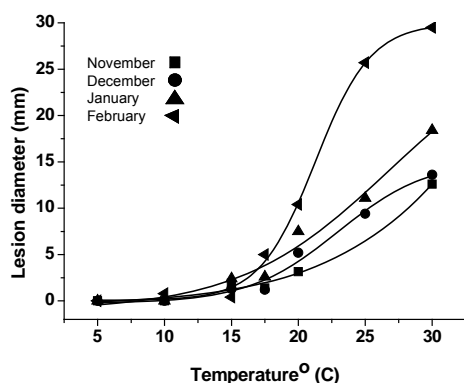


Figure 1. Mean lesion diameter (mm) of detached apple fruit inoculated at harvest with 10^6 spores/ml *Colletotrichum acutatum*. A Boltzmann plot was fitted.

RESULTS

Detached ‘Royal Gala’ apples were susceptible to infection by *C. acutatum* when a temperature of c. 15°C was exceeded, regardless of maturity (Fig. 1). A wetness period of 72 hours was required for infections without wounding (results not shown). In the field ‘Royal Gala’ apples were infected by *C. acutatum* after a temperature of 15.2°C was exceeded (Fig. 2).

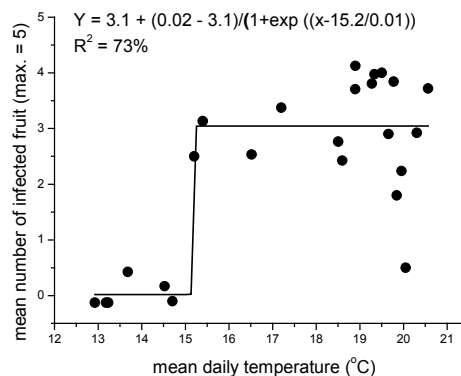


Figure 2. The mean number of infected apple fruit out of five inoculated with 10^6 spores/ml of *Colletotrichum acutatum* plotted against the mean daily temperature for 72 hours after inoculation. A Boltzmann plot was fitted. The x0 value (inflection point) = 15.2°C.

DISCUSSION

In New Zealand mean daily temperatures of 15°C are exceeded during December, January and February. Effective control without residues may be able to be achieved by reducing inoculum early in the season before temperatures are above mean daily temperatures of c. 15°C, as was achieved for control of *B. dothidea* (2). A biological control agent or a benign chemical such as calcium chloride (3) could be used to protect the fruit from infection during summer when temperature is not limiting infections.

ACKNOWLEDGEMENTS

This work was funded by MAF Sustainable Farming Fund, Pipfruit New Zealand, Waikato Fruitgrowers’ Association and Nelson Group 8.

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Epidemiology of walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis*, in Tasmania, Australia

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INTRODUCTION

Walnut blight is an important bacterial disease of walnuts world-wide, and can cause the premature drop of almost all fruit in some Australian orchards (1). In glasshouse trials, only five minutes of continuous wetness on the fruit surface is necessary for infection by *Xanthomonas arboricola* pv. *juglandis* (2), and extensive wetness periods may explain polycyclic disease epidemics in California (3). Objectives of this study were to characterise the temporal progression of disease incidence and severity in walnut fruit in Tasmania, Australia, and the relationship between disease severity and yield of marketable nuts.

MATERIALS AND METHODS

Randomised, complete block trials were conducted on single tree plots ($n = 6$) of Vina and Franquette over three and two growing seasons, respectively, in northern Tasmania. On non-treated trees, disease incidence and severity was assessed on the same 100 fruits from fruit set to harvest, or until a fruit fell. Severity was estimated visually as the percent area of the fruit covered with blight symptoms. Incidence was derived from the severity data as the percentage of fruit with symptoms. Daily cumulative disease incidence was analyzed as a function of time with linear, monomolecular, exponential, logistic and Gompertz models. Crop yield in non-treated plots was defined as the percentage of the 100 fruit present at fruit set that produced marketable nuts at harvest.

RESULTS AND DISCUSSION

The time of disease onset was similar for each epidemic whereas the rate of disease increase was highest in 2005–06 with nearly 100% incidence within 80 and 120 days of bud-burst in Vina and Franquette respectively (Fig. 1). Temporal progression of disease incidence was described well by the monomolecular model in 2004–05 and 2006–07, implying monocyclic disease epidemics (Table 1). In contrast, the logistic and Gompertz models described putative polycyclic disease in Vina and Franquette, respectively, in the relatively wet season of 2005–06. Disease severity of fruits at half full-size diameter accounted for 74% of the variation in crop yield (Fig. 2). No marketable nuts were predicted when the mean blight severity on fruit was 10%. In contrast, 87% of fruits were predicted to produce marketable nuts when the mean blight severity was 2%.

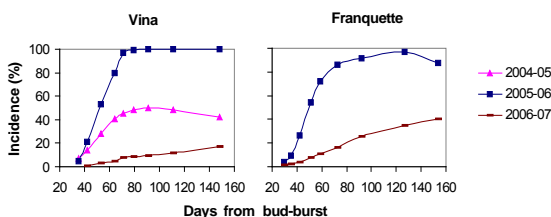


Figure 1. Temporal progression of disease incidence observed on Vina and Franquette fruits in Tasmania.

Table 1. Linear models of the temporal progression of walnut blight incidence in Vina and Franquette in Tasmania.

Year	Model	R ² ^A	Intercept	Slope	Day 50% ^B
<i>Vina</i>					
2004-05	Monomolecular	0.9677	-0.3203	0.0121	84
2005-06	Logistic	0.9922	-10.0603	0.1926	52
2006-07	Monomolecular	0.9886	-0.0655	0.0018	-
<i>Franquette</i>					
2005-06	Gompertz	0.9926	-3.5383	0.0771	51
2006-07	Monomolecular	0.9946	-0.1298	0.0042	-

^A Back transformed R², ^B Days (predicted) to 50% disease incidence.

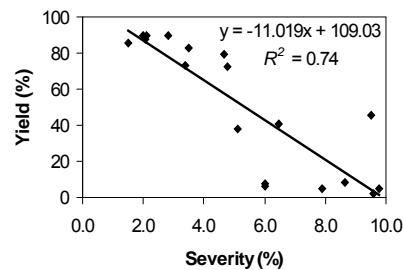


Figure 2. Yield per plot of Vina nuts at harvest, expressed as a percentage of 100 fruit at fruit set, and disease severity observed on fruits at half full-size diameter.

DISCUSSION

In Tasmania, epidemics of walnut blight appear to be either monocyclic or polycyclic, with polycyclic epidemics leading to all fruits developing disease symptoms. Preventing the onset and rate of increase of disease incidence and severity of fruits prior to fruits attaining half full-size diameter appears critical to reducing crop loss. An empirical, weather-based model for timing applications of copper is being developed.

ACKNOWLEDGEMENTS

This project was supported by the Australian Government through Horticulture Australia Limited in partnership with Webster Walnuts, and was managed by Agronico P/L., 175 Allport St. East, Leith, TAS, 7315 www.agronico.com.au

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Sugarcane smut—disease development and mechanism of resistance

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INTRODUCTION

Sugarcane smut caused by *Ustilago scitaminea* is an important disease worldwide. This disease can be effectively managed by replacing susceptible cultivars with resistant cultivars. The current smut rating system relies on artificial inoculation and a short plant and ratoon crop Ratings are based on % infected plants, not taking into account the severity of the disease.

Infection of smut occurs through germinating or dormant buds of standing stalks or through germinating buds in the soil (1). Available literature suggests that there are two mechanisms of resistance to smut: i) bud scale or external resistance; and ii) internal resistance (2). Bud scale resistance is believed to be a combination of physical and chemical barriers to infection, and internal resistance is governed by interactions within plant tissue. It is also suggested that buds become increasingly resistant with age.

The objectives of the study were to: i) monitor development of sugarcane smut; ii) compare disease incidence and severity; and iii) determine mechanisms of disease resistance of important sugarcane cultivars grown in Australia.

MATERIALS AND METHODS

Disease development. Twenty-nine commercial cultivars were collected from various regions of Queensland. They were cut into one-eye-setts, inoculated by dipping in a spore suspension (10^6 spores/mL) and planted in September 2008 in 3 replications using a randomised complete block design. Disease incidence and severity were measured each month after planting.

Mechanism of resistance. A range of smut susceptible, intermediate and resistant cultivars were selected for the trial. Ten buds were cut from each stalk, separated and grouped into five batches, so the two oldest buds were placed in group 5, next oldest in group 4 and so on. The setts were inoculated using one of the two inoculation methods, i) dip in smut spore suspension or ii) inject smut spore suspension into the base of the bud. After germination the inoculated seedlings were transplanted in the field using randomised complete block design. Per cent smut-infected plants were assessed each month.

RESULTS AND DISCUSSION

Disease development. Initial results indicated a strong correlation between incidence and disease severity (Figure 1). Some cultivars would receive a lower rating if the ratings were based on disease severity compared with disease incidence. The trial will be ratooned in September 2009, and disease assessment of ratoon crop will continue until September 2010.

Mechanism of resistance. Mean disease incidence in inject inoculated treatment (40%) was significantly ($P < 0.0001$) higher than that of dip inoculated (15%) treatment. Clear indications of bud and internal resistance were evident within cultivars (Table 1). Younger buds were more smut susceptible than older buds (Figure 2). This result suggests that with age, buds become increasingly resistant to smut.

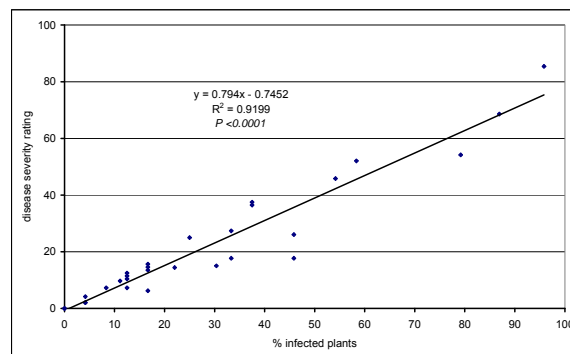


Figure 1. Correlation of smut disease severity and disease incidence of 29 cultivars.

Table 1. Smut resistance mechanism of some selected cultivars

Cultivar	Smut rating	Smut%		Res. Mech.
		(Dip)	(Inject)	
KQ228 ^A	2	7.5	60	Bud
Q99	1	2.5	45	Bud
Q171 ^A	1	0	7.5	Internal
Q232 ^A	3	0	4	Internal
Q135	5	2.5	52.5	Bud
Q190 ^A	4	5	22.5	Bud
Q96	5	2.5	47.5	Bud
NCo310	8	57.5	77.1	None
Q141	9	70	92.5	None
Q205 ^A	9	58.2	92.5	None

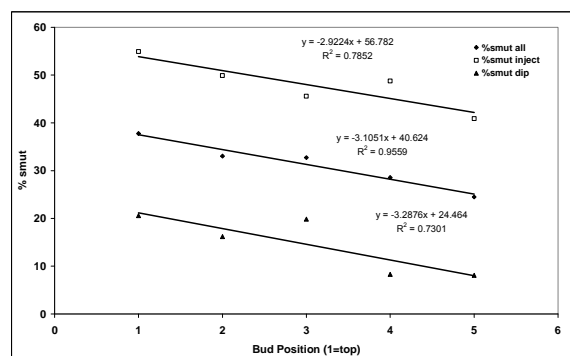


Figure 2. Smut incidence on sugarcane plants derived from various sections of stalks (5 = oldest buds and 1 = youngest buds)

ACKNOWLEDGEMENTS

Sugarcane Research and Development Corporation (SRDC) funded this research work.

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Dissemination of biological and chemical fungicides by bees onto *Rubus* and *Ribes* flowers

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INTRODUCTION

Honey and bumble bees have shown to be capable of disseminating biological fungicides (BF) to achieve inoculation of berry flowers, such as strawberry, blueberry and blueberries. This in turn has caused a reduction in flower-borne diseases. The aim of our work was to study the efficacy of a BF (year Y1) and a chemical fungicide (CF) (Y2) disseminated by honey bees and bumble bees (Y2 only) onto boysenberry (*Rubus* hybrid) and blackcurrant (*Ribes nigrum*) flowers.

MATERIALS AND METHODS

- BF: Sentinel® (Trichoderma atroviride)
- CF: Switch® (fludioxonil+cyprodinil) finely ground and mixed with fluorescent dye (1:1, v/v)
- Honey bees (*Apis mellifera*) and bumble bees (*Bombus terrestris*) at approx. 25000 and 250 bees/hive, respectively were used.
- All experiments were conducted on commercial boysenberry and blackcurrant fields, with 2–4 replicate grower sites per crop and product.
- The study was conducted separately for BF and CF over two flowering seasons. In Y1, honey bee and BF dissemination was examined. In Y2, honey and bumble bee CF disseminations were monitored, including CF residues in honey.

RESULTS AND DISCUSSION

Y1: Application efficacy (% flowers inoculated) of bee-applied Sentinel® was similar to spray-applied BF. Irrespective of application method in boysenberry, 60–80% of flowers were inoculated; in blackcurrant, 40–50%. This resulted in >160 *Trichoderma* colony forming units or cfu/boysenberry ovary and 3–4 cfu/blackcurrant style. Bee application could be maximised by either equipping all hives with dispensing units and/or by increasing the release rate from three per week to daily releases. The BF, bee- or spray-applied, did not improve disease control. Therefore in Y2, a CF was selected.

Y2: CF or dye recovery on blackcurrant flowers was in the order of 5–14% for honey bees and 5–9% for bumble bees. The average number insects foraging per 10-m row was 5 honey bees and 0.1 bumble bees. The average number of bees exiting the experimental hives during 1 and 5 min of observation was 300 honey bees and 3.4 bumble bees, respectively. Bumble bees visited similar numbers of flowers as the honey bees, although there were 50 times more honey bees active in the crop than bumble bees. In addition, there were only 5 bumble bee hives in the blackcurrant field (>5 ha) compared with 14 honey bee hives for the same area (only two hives were equipped with the dispensers).

In boysenberry, 2% and 1.3% of the flowers were visited by honey bees and bumble bees respectively, carrying Switch®+dye. The number of total honey bees (46) and bumble bees (0.7) observed was 0.5 honey and 0.08 bumble bees foraging on one boysenberry plant, with a daily average of 70–80 open flowers/plant available. Bumble bee hive activity was, however, very low, with 0.05 bumble bees leaving the hive/15 minutes. Approximately 5% of honey bees exposed to CF+dye vectored the fungicide to 2–4% of flowers. The product mixture probably was only available during approximately 15% of the actual foraging time for those bees. We can conclude that honey bees can vector biological and chemical fungicides to boysenberry flowers, however, in the level of disease control is not yet established. The role of bumble bees in boysenberry gardens is inconclusive, as the hive activities were very low.

Switch® residues in honey from hives fitted with fungicide dispensers collected immediately after flowering were up to 5 mg/kg active ingredient. No fungicide residues were measured in honey samples from adjacent hives, but without CF dispensers.

ACKNOWLEDGEMENTS

The work was funded by The New Zealand Boysenberry Council Ltd, Blackcurrant New Zealand Ltd and the Sustainable Farming Fund (SFF) of the Ministry of Agriculture and Forestry (MAF) Grant 06/007.

Current studies on divergence and management of pepper yellow leaf curl disease in Indonesia

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INTRODUCTION

Whitefly-transmitted geminiviruses (WTGs) has been reported to infect several crops in Indonesia including tobacco, tomato, chilli pepper, ageratum, and cucumber. Infection of WTGs in chilli pepper causes severe crop damage and becoming a major threat since early 2000. The most unique symptoms associated with the virus infection involved yellowing and leaf curling, therefore it was known as pepper yellow leaf curl (PYLC) disease. Biological and molecular characterisation of the causal agent reveals that several WTGs are associated with the disease. Disease spread was very fast due to activity of its insect vector, *Bemisia tabaci*, which grows very prominently in the tropic climate. Therefore, disease control is becoming very difficult. Breeding program for WTGs resistance varieties is one of major activities in regard to disease control strategy in Indonesia since commercial cultivars carrying resistance to the diseases have not yet been released. Evaluation of chilli pepper genotypes showed that some germplasms are very promising for development of cultivars with resistance or tolerance to the disease.

MATERIALS AND METHODS

Analysis of Genetic Diversity. Pepper plant showing typical symptoms of PYLCV infection were collected from several chilli pepper production areas in Indonesia. Extraction of total DNA and PCR amplification was done according to procedure explained previously (1, 2). Sequence data obtained following nucleotide sequencing of the PCR product was analysed using ClustalW program version 1.83 EMBL-EBI.

Evaluation of Chilli Pepper Genotypes. Inoculation of PYLCV by *B. tabaci* was conducted as explained previously (3). Response of different chilli pepper genotypes was classified into three groups i.e. resistant, moderately resistant, and susceptible based on symptoms expression and disease incidence.

RESULTS AND DISCUSSION

Identity of geminivirus infecting chilli pepper in Indonesia was determined based on their hairpin loop structure and repetitive sequence found in the common region. These hairpin loop structure was found in all geminivirus sequences so far (4). Variability in the structure as well as the length of hairpin loop region was observed among PYLCV isolates. This may indicate the possible genetic diversity among WTGs infecting chilli pepper in Indonesia. Phylogenetic analyses involving 32 sequences showed that PYLCV isolates can be differentiated into several clusters. Interestingly, they are all quite different from WTGs infecting tomato in Indonesia but more closely related to tomato yellow leaf curl virus from Thailand.

Evaluation of 11 commercial cultivars and 27 genotypes of chilli pepper showed that the symptoms were developed within 2 to 3 weeks after inoculation, although some genotypes required longer incubation period. Disease incidence was varied among different genotypes, i.e. in the range of 12 up to 100%. Selection of potential genotypes was proceeded for further breeding activity in order to develop resistant varieties for PYLCV.

ACKNOWLEDGEMENTS

This research was supported in part by ACIAR—AVRDC Chilli Integrated Disease Management Project.

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Fungicide resistance in cucurbit powdery mildew

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INTRODUCTION

Powdery mildew, caused by *Podosphaera xanthii*, (Castagne) is a major constraint to commercial cucurbit production in Australia and worldwide. Management of this disease has relied primarily on the use of foliar fungicides sprays. The development of strains of the pathogen with resistance to systemic fungicides is becoming increasingly widespread with the excessive use of these fungicides. Resistance problems were first reported in Queensland in the late 1980s (1) and since then there has been no resistance monitoring program.

The aim of this study was to determine if resistance had developed to the four systemic fungicides (Amistar, Bayfidan, Nimrod and Spinflo) registered in Australia for the control of powdery mildew in cucurbit crops in the Burdekin region of north Queensland.

MATERIALS AND METHODS

In 2008, 21-day old seedlings of a powdery mildew susceptible zucchini variety (Congo, SPS) were used in bioassays to test for resistance against current registered fungicides. Plants with good vigour were sprayed with the four systemic fungicides at half, full and double the recommended label rates of application and water as controls, 24 h prior to overnight field exposure in various cucurbit crops at seven different locations in the Burdekin production region. Actively growing apical shoots were removed from each plant leaving two cotyledons and three to four true leaves.

The exposed treated seedlings were randomly placed on benches in a glasshouse where night temperatures averaged about 20°C and day temperatures 30°C. Three lower true leaves of each seedling, which served as replications for each plant were rated for disease severity 11 and 15 days after field exposure. Disease severity (% leaf area infected) was estimated to the nearest 5%. The data collected was analysed using Genstat 11 to determine treatment differences.

RESULTS

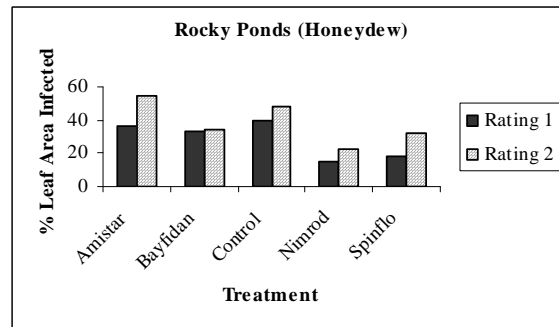
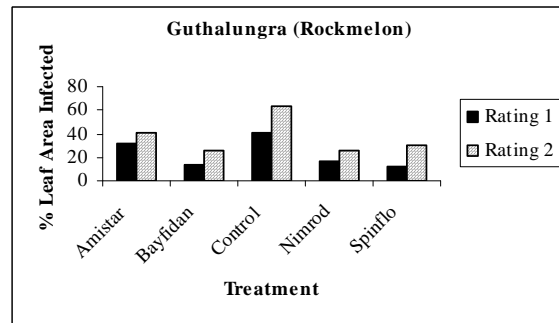
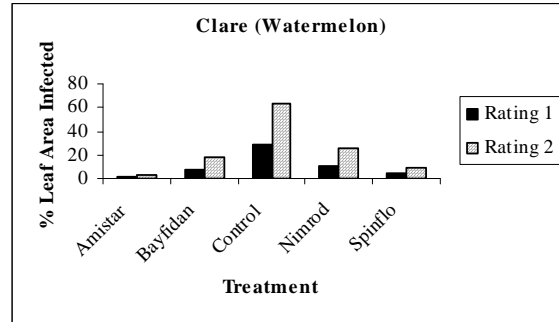
Powdery mildew infection was first noticed on the water-sprayed control seedlings 7 days after field exposure. Disease severity at the second rating was always higher than the first (Fig 1; A-C; based on the recommended label rate for each of the fungicides). There was low disease severity ($\leq 15\%$ on the controls) at four of the locations with no significant treatment differences for the first and second ratings. At the other three locations; Clare, Rocky Ponds and Guthalungra, disease severity on the controls was quite high (~60%). All the fungicide treatments were effective against the disease, except at Rocky Ponds and Guthalungra where they were not significantly different from the controls.

DISCUSSION

The results from Rocky Ponds and Guthalungra clearly show that there is a fungicide resistance problem in some areas in the Burdekin region. This is a major cause for concern. Similar results were recorded on seedlings exposed to isolates from the Bundaberg region of Central Queensland.

These results reinforce the need for monitoring of isolate sensitivities to the main systemic fungicides on a seasonal basis

in the main production regions. Promoting integrated crop management strategies is vital. These include spraying only when needed, using resistant varieties and using fungicide alternatives as substitutes for protectant fungicides, as well as destroying old crop residues in finished strips.



Figures 1. A-C: Effect of systemic fungicides on powdery mildew disease severity in cucurbit crops in the Burdekin region of north Queensland.

ACKNOWLEDGEMENTS

Funding for this work was provided by Horticulture Australia Limited (HAL) for which we are grateful.

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Population genetic analyses of plant pathogens: new challenges and opportunities

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INTRODUCTION

The study of population genetics attempts to investigate evolutionary forces such as mutation, migration, genetic drift, selection and recombination, and how gene frequencies change in populations to shape their genetic structure. These evolutionary forces and the interaction amongst them are particularly important in plant pathogens where, combined with the pathogen's life history characteristics, they determine the evolutionary potential. The population genetics of plant pathogens has been investigated for at least 30 years. Early studies on population genetics of plant pathogens concentrated on the effect sexual reproduction has on levels of genetic diversity in populations (Burdon and Roelfs, 1985a, b) and what impact that had on disease control. Similar studies have continued with investigations of pathogen capacities to rapidly adapt to new environments such as developing resistance against a fungicide or overcoming a resistance gene in the plant host (McDonald and Linde, 2002).

Although the questions we ask in the population genetics of plant pathogens has not changed significantly, advances in DNA sequencing and analytical approaches have significantly improved the accuracy of parameter estimates. In particular, coalescent based approaches are a powerful extension of classical population genetics because it is a collection of mathematical models that can accommodate biological phenomena as reflected in molecular data. The emphasis in coalescent thinking is to view populations backwards in time, using the divergence observable in a population to estimate the time to a most recent common ancestor. This ancestor is the point where gene genealogies 'coalesce', in a single biological organism.

The barley scald pathogen, *Rhynchosporium secalis*, will be used as an example to illustrate the importance of some of these evolutionary forces and how coalescent based methods significantly improved our understanding of the pathogens' biology.

MATERIALS AND METHODS

Populations of *R. secalis* were characterised with 14 microsatellite loci (Linde et al., 2009) and several sequence loci (Zaffarano et al., 2009). Several population genetic parameters were investigated, including migration among populations. This was investigated with a coalescent method in the program IM (Hey and Nielsen, 2004) and results were compared to estimates derived from traditional F_{ST} estimates (Weir and Cockerham, 1984).

RESULTS AND DISCUSSION

The results of this comparison revealed that coalescent based approaches offer several advantages over other analytical methods to estimate parameters such as migration and genetic drift. Traditional measures of the translation of F_{ST} into gene flow assume that subpopulations have the same size, population sizes are constant, or that there are infinitely many populations, and that migration rates are all symmetric. Due to these underlying assumptions, migration estimates derived from F_{ST} are almost always flawed and incorrect estimates are achieved when these assumptions are not met. This is often the case since

pathogen populations are constantly influenced by the host populations or human-mediated migration.

With coalescent methods, the direction of migration is obtained. This means the major source and sink populations for migration can be determined which is useful in determining breaches of quarantine or major migration routes due to eg prevailing wind currents. In *R. secalis*, unusually high migration rates in both directions between Australia and South Africa and Australia and New Zealand cause particular concern for disease management.

A comparison of the results revealed that migration estimates based on coalescent analyses were frequently non-symmetric, meaning one population contributed significantly more migrants than the other. This contributed to migration estimates derived from F_{ST} being over- or under-estimated. Furthermore, F_{ST} derived migration estimates were usually underestimated when the migration was high, and/or when population sample sizes were low.

Coalescent analyses provided population genetic parameter estimates that are more reliable, are less dependent on population sizes being stable and are affected less by populations with small sample sizes. Improved analyses and their usefulness in plant pathology are discussed.

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Genetic diversity of *Botryosphaeria parva* (*Neofusicoccum parvum*) in New Zealand vineyards

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INTRODUCTION

Species of the Botryosphaeriaceae cause disease on numerous woody and non-woody hosts. Worldwide several species of *Botryosphaeria* have been reported to cause various symptoms on grapevine including decline and die-back. In a survey carried out in 2007/08, six *Botryosphaeria* species were isolated from symptomatic New Zealand grapevines. Among these *Botryosphaeria* species, *Neofusicoccum parvum* was identified as the most prevalent species (1). The aim of this study was to analyse the genetic diversity of the *N. parvum* isolates collected from the New Zealand vineyards and to compare these with international isolates.

MATERIALS AND METHODS

Fungal isolates and DNA extraction. New Zealand isolates of *N. parvum* (49), collected from six grape growing regions as well as three Californian and four Australian isolates were used in this study. The identity of the New Zealand isolates was confirmed by using a published PCR-RFLP (ARDRA) method (2). Genomic DNA was extracted from mycelium using the PUREGENE[®] genomic DNA isolation kit and concentration was adjusted to 20 ng / μ l for UP-PCR.

UP-PCR procedure. For genetic variation analysis of *N. parvum* isolates, 11 UP-PCR primers (3) were tested, of which five (AA2M2, Fok1, L15, 0.3-1 and 3-2) were chosen for further use, based on total number of bands, number of polymorphic bands and the band distribution. UP-PCR amplification products were separated by electrophoresis on a 1% agarose gel for 3 h at 100 V.

Genetic variation analysis. Bands were counted if they were strong and reproducible. A binomial matrix was produced as follows: if a band was present it was indicated by a "1" and if absent by a "0". The binomial matrix thus generated was used for phylogenetic analysis using PAUP version 4.0b10. A neighbour joining cladogram was generated to show the relationships between the genotypes produced for all 56 isolates.

Pathogenicity test. From each general branch of the *N. parvum* neighbour joining tree, two isolates were selected (15 isolates in total) for *in-vitro* green shoot assays. Shoots of similar age and diameter, 25–30 cm long, were cut from glasshouse grown Sauvignon Blanc plants. They were prick-wounded and inoculated with mycelium colonised agar plugs from 3 day old cultures, wrapped onto the wounds with parafilm. Inoculated shoots were arranged randomly in a growth chamber with five replicates for each isolate. Pathogenicity of isolates was assessed as the external lesion lengths, measured at 7 d post inoculation. The data were analysed using ANOVA (GenStat 11) Means were separated by Fisher's protected least significance difference (LSD) test.

RESULTS

The 61 informative bands produced with the five UP-PCR primers were used to compile a neighbour joining tree. This tree revealed a high degree genetic variability in the *N. parvum* populations studied. Genetic variability was higher for inter-

vineyard rather than intra-vineyard populations. Only one of the four Australian isolates grouped together with New Zealand isolates. All of the Californian isolates were grouped into a branch with three New Zealand isolates collected from a single region. The *N. parvum* isolates selected from different branches of the neighbour joining tree differed significantly ($P < 0.001$) in their pathogenicity on green shoots (Table 1). The largest lesion of 82.5 mm was caused by isolate G22a3 and the lowest of 11 mm by isolate A421.

Table 1. Mean lesion length produced on grapevine green shoots by *N. parvum* isolates with their region of origin and genetic grouping.

Isolate	Region	Genetic group	Mean lesion length
A421	Auckland	8	11.00 a ¹
G61a1	Gisborne	2	15.25 a
Nu3	Auckland	8	20.00 a
G652	Gisborne	7	27.50 ab
B2141	Blenheim	9	27.75 ab
G121	Gisborne	7	28.75 ab
A102B6	Auckland	4	38.00 abc
A842	Auckland	5	41.25 abcd
G61c1	Gisborne	5	51.25 bcd
B31612	Blenheim	6	55.00 bcde
MM562	Hawke's Bay	6	56.25 bcde
A122	Auckland	4	60.25 cde
A75a1	Auckland	3	66.25 cde
I(15)3	Nelson	3	70.00 de
G22a3	Gisborne	1	82.50 e

¹Means with the same letter are not significantly different from each other ($P < 0.001$)

DISCUSSION

This analysis indicated that the New Zealand *N. parvum* population has a high degree of genetic variability and that some isolates were genetically distinct from the Australian and Californian isolates tested. This high degree of genetic variation was unexpected as sexual reproduction of *Botryosphaeria* species is rarely observed in the field (4). However, in this study no sexual or asexual reproductions have been discovered in the fields. Further detailed study needs to focus on what mechanisms are present within this species to generate genetic diversity. Isolates from different branches in the neighbour joining tree showed different levels of pathogenicity indicating a genetic basis for its disease producing capacity.

ACKNOWLEDGEMENTS

The authors wish to thank the New Zealand Winegrowers association for funding this project.

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Anthracnose disease of chili pepper—genetic diversity, pathogenicity and breeding for resistance

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INTRODUCTION

Anthracnose disease of chili pepper (*Capsicum annuum*) is caused by a complex of *Colletotrichum* species with *C. capsici*, *C. acutatum*, *C. gloeosporioides*, being the most severe pathogens in SE Asia and Australia (1). Elucidation of the disease cycle for *C. capsici* indicated that quiescent leaf infection was an important source of inoculum thus necessitating more efficient use of fungicides to prevent fruit infection.

MATERIALS AND METHODS

Genetic Diversity: The genetic structure of populations of *C. capsici* collected from Australia, India, Sri Lanka and Thailand were analysed using loci-specific microsatellite markers (2) The number of effective alleles and the genetic diversity was determined using genalex 6 software.

Pathogenicity: Differential reactions based on qualitative host reactions (infection vs no infection) on mature green and ripe chili fruit of 10 genotypes from four cultivated *Capsicum* species—*C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens* were investigated after being inoculated with 33 isolates of *C. capsici*, *C. gloeosporioides* and *C. acutatum* from Thailand. Bioassay and host reaction was recorded as described in Montri et al. (2009).

Breeding for Resistance: Resistance to anthracnose caused by *Colletotrichum capsici* and *C. acutatum* was investigated in *C. chinense* PBC932 and *Capsicum baccatum* PBC80 and PBC1422. Mature green and ripe fruit were inoculated with 13 isolates of the two *Colletotrichum* species. Bioassay and host reaction was recorded as described in Montri et al. (2009).

RESULTS AND DISCUSSION

Genetic Diversity: Screening of 117 isolates against 27 STMS markers revealed 92 haplotypes and a total of 148 alleles present across all the populations. A highly significant population differentiation (0.154) was found among the populations. The Australian population was relatively homogeneous with low level of gene diversity and high population differentiation suggesting their recent origin (probably caused by genetic bottlenecks) in comparison with highly diverse Indian, Sri Lankan and Thai isolates. The overall high gene flow and diversity indicated that *C. capsici* had a high adaptive potential to overcome control measures such as host resistance and fungicides.

Pathogenicity: Differential reactions on mature green and ripe chili fruit of 10 genotypes of cultivated *Capsicum* spp identified 5, 11 and 3 pathotypes of *C. capsici*, *C. gloeosporioides* and *C. acutatum* respectively. This will have profound effect on chili breeding programs where novel sources of resistance genes from related species are being incorporated into commercial *C. annuum* varieties. Putative PR genes have been identified through transcriptional analysis from a virulent pathotype of *C.*

capsici and an *Agrobacterium*-mediated fungal transformation system developed for assessing function of these genes.

Breeding for Resistance: The resistant *C. chinense* genotype PBC932 showed a strong hypersensitive response to infection by *C. capsici* pathotypes. Resistance was found to be controlled by three recessive genes at specific growth stages. This resistance is being incorporated into commercial varieties through marker assisted selection. ie *co1* at mature green fruit, *co2* at ripe red fruit, and *co3* at seedling stages of plant growth. Linkage analysis suggested that *co1* and *co2* were linked (recombination frequency 0.25), and that the *co3* was not linked to the fruit resistances. Resistance at mature green fruit stage in *C. baccatum* to *C. acutatum* was found to be controlled by a single recessive gene *co4* and at ripe fruit stage by a single dominant gene *Co5*. Linkage analysis between the two genes showed the genes to be independent. Markers to these genes will be developed for use in Marker Assisted Selection to enable the development of highly resistant chili varieties.

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The diversity of *Colletotrichum* infecting lychee in Australia

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INTRODUCTION

Pepper spot caused by *Colletotrichum gloeosporioides* is a relatively new disease of lychee (*Litchi chinensis* Sonn.) in Australia. While the disease only causes superficial damage to the skin of the fruit it does result in lower financial returns to lychee growers (1). Unlike anthracnose of lychee, symptoms of pepper spot develop prior to harvest with out an apparent quiescent period for *C. gloeosporioides*. Host stress and environment have been suggested to contribute to pepper spot development; however it is not clear if the genotype of *C. gloeosporioides* affects pepper spot development. In this study we compared isolates of *Colletotrichum* spp. from anthracnose and pepper spot lesions on lychee to determine if a new genotype of *Colletotrichum* sp. is responsible for pepper spot of lychee.

MATERIALS AND METHODS

Collection and characterisation of isolates. One-hundred and fifty isolates of *C. gloeosporioides* were collected from pepper spot and anthracnose lesions of lychee from three orchards in eastern Australia. Isolates derived from single germinated conidia were characterised on the basis of morphology (conidia shape and size, production of teleomorph) and molecular fingerprint using arbitrary-primed PCR (ap-PCR) using two primers.

Field pathogenicity testing. Thirteen isolates representing the main genotypes identified using ap-PCR were selected for field pathogenicity testing. An isolate of *C. gloeosporioides* (BRIP28734) from mango was included in the pathogenicity testing. Lychee fruit were inoculated two weeks prior to harvest and were assessed for the development of pepper spot at harvest and for the development of anthracnose after 10 days of storage at 20°C.

RESULTS AND DISCUSSION

Collection and characterisation of isolates. Of the 150 isolates in the collection, nine were *C. acutatum*, isolated from anthracnose lesions. The rest of the isolates were *C. gloeosporioides*. Analysis of conidial measurements did not indicate a distinct sub-population within *C. gloeosporioides* responsible for the development of pepper spot. The production of the teleomorphic stage was not limited to isolates from one symptom type or location.

The ap-PCR analysis did not differentiate the pepper spot isolates from the anthracnose isolates. The majority of the isolates (73%) grouped together with 75% similarity to each other. Within this group were isolates from both pepper spot and anthracnose as well as isolates from all three locations.

Most other genotypes identified had only one or very few isolates, except for a genotype which contained 15 isolates from the same location. Of the latter, 14 isolates produced the teleomorph under laboratory conditions. Both pepper spot and anthracnose isolates were in this group.

Field pathogenicity testing. Only isolates ALPS11, ALAN11 and RLPS24 caused significant levels of pepper spot (Figure 1), all of these isolates were from the closely related group identified using ap-PCR which contained the majority of isolates. The other isolates from this group ALPS15, ALAN12 and RLAN11 did not cause pepper spot but did cause high levels of anthracnose after storage. None of the isolates in the large closely related group produced the teleomorph in culture, unlike the isolates of *C. gloeosporioides* from other genotypes of which 60% produced the teleomorph.

Fruit inoculated with the mango isolate (BRIP 28734) had levels of anthracnose similar to the uninoculated control. Of the lychee isolates, GLPS12 caused only low levels of anthracnose which were not significantly different to those of the mango isolate. GLPS12 was from the genotype where the majority of the isolates produced the teleomorph. It is possible that this group of isolates is not specific to lychee.

On the basis of ap-PCR and morphological studies there were no apparent differences found between pepper spot and anthracnose isolates. However, when assessed together, the pathogenicity testing and molecular work suggest that there may be a group of isolates more likely to cause pepper spot. To confirm the host specificity of the isolates cross pathogenicity testing is being conducted with a range of hosts.

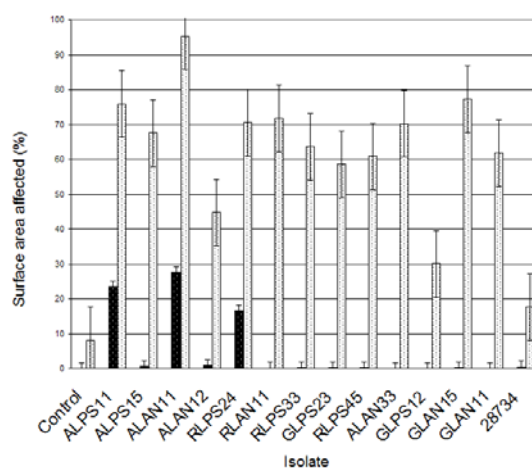


Figure 1. Preharvest pepper spot (solid columns) and postharvest anthracnose (white columns) development on lychee fruit inoculated 2 weeks prior to harvest. Error bars indicate standard error.

ACKNOWLEDGEMENTS

JA is recipient of a DAFF Science and Innovation Award

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Variation in *Phytophthora palmivora* on cocoa in Papua New Guinea

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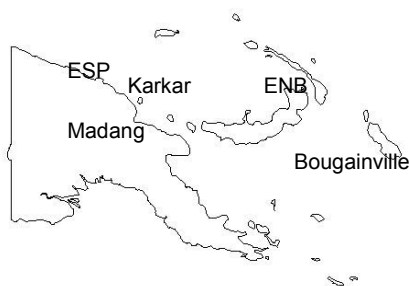
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INTRODUCTION

Phytophthora palmivora is the major cause of cocoa disease in PNG, although *P. arecae*, *P. megakarya*, *P. nicotianae*, and *P. citrophthora* have also been suggested as pathogens. The success of disease control strategies depends on a thorough knowledge of the pathogen and its population biology. Furthermore, all cocoa planting material is bred in ENB and distributed throughout the country, without any comprehensive

This study tested the hypotheses that: 1. *P. palmivora* is the sole *Phytophthora* sp. causing disease on cocoa in PNG; and 2. there is variation between *P. palmivora* populations from different cocoa growing locations in PNG.

MATERIALS AND METHODS



Sampling locations in PNG

Figure 1. Sampling locations in PNG

Diseased pods were sampled hierarchical from 5 locations (Fig 1); 8 farms/location, 8 diseased pods/farm. Isolates were grown on carrot agar in the dark at 25°C for 4 days and growth rates taken simultaneously with colony morphology. For sporangial morphology, isolates were grown as described for 10–14 days and sporangial length, breadth and pedicel length of fifty sporangia per isolate measured and length/breadth ratio calculated. Sporangiphore branching and caducity were also recorded. Mating type was determined using Duncan's media (3). Genetic variation was studied using Randomly Amplified Microsatellite (RAMS) analysis. Genomic DNA was extracted and amplified by PCR and PCR products separated by agarose gel electrophoresis. Loci with clear bands only were scored.

RESULTS

Colony morphology of the isolates were stellate striate. Sporangiphore branching was simple sympodium and sporangia were caduceus. Mean Sporangia length, breadth, pedicel length and length:breadth ration were 50.1µm, 27.2µm, 4.7µm and 1.8 respectively. Growth rates were not correlated with locations. Sporangia length, breadth and length:breadth ratio were different depending on locations. Both mating types A1 and A2 are present in PNG. Genetic analysis revealed seven clonal groups (Figure 2).

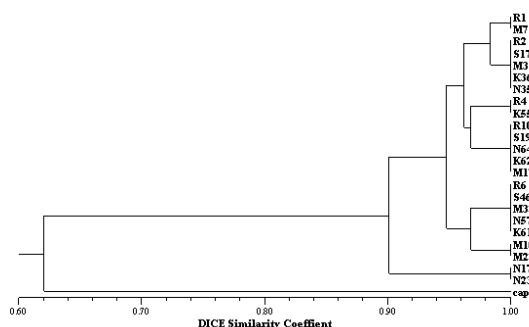


Figure 2. UPGMA dendrogram based on RAMS analysis, representatives of 263 isolates of *P. palmivora* from PNG including references, *P. capsici* (Caps).

DISCUSSION

Isolates displayed striate/stellate colony morphology which is typical of *P. palmivora* (2). Sporangiphore branching, caducity and sporangial dimensions agree with descriptions of *P. palmivora* (4). Sporangia pedicel length was intermediate (~5µm), typical of *P. palmivora* (1). RAMS analysis clearly separated *P. capsici* from the PNG isolates and revealed that the population in PNG was generally clonal with emerging sub-populations in Bougainville and Madang. Both mating types A1 and A2 are present in Madang posing a high risk of variation. *P. palmivora* is the sole *Phytophthora* sp causing disease on cocoa in PNG. The presence of a single species means concentrating cocoa breeding in one location is acceptable; however mixed cocoa cultivars should be deployed and integrated disease management promoted. Strict quarantine should be imposed, especially in Madang where both mating types are present.

ACKNOWLEDGEMENTS

The scholarship provided by AusAid to undertake this study is highly appreciated. The assistance of CCIPNG staff for isolate collection and assistance provided by Dr Rose Daniel at The Sydney University is acknowledged.

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Spore traps for early warning of smut infestations in Australian sugarcane crops

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INTRODUCTION

Sugarcane is Australia's second most important agricultural export crop on a monetary basis, being grown on over 400,000ha of land to produce >30m tonnes of harvest product and >3m tonnes of crystal sugar. There have been many climatic, market and disease threats to the industry and recently sugarcane smut (*Ustilago scitaminea*) has posed a major threat. In 2006 the disease was found for the first time in the major eastern seaboard sugarcane production area. Previous research showed that the majority of the Australian commercial cultivars were highly susceptible to the disease; rapid spread and escalation of the disease therefore threatened crop yields and profitability of the Australian sugarcane industry. As the disease is difficult to find in mature crops, and as early warning of the disease would provide the greatest opportunity for farmers to transition to more resistant cultivars, it was decided to use atmospheric spore trapping as an early warning tool. This paper describes how spore trapping has provided early warning of the presence of smut to the Australian sugarcane industry.

MATERIALS AND METHODS

Smut outbreak. Smut was found for the first time in the Childers region in June 2006, then shortly afterwards in the Mackay production area in November 2006 and in the Herbert River district in December 2006. Early warning research was principally applied therefore to other production areas: these included northern Queensland (Tully north), the Burdekin Irrigation Area, specific parts of central and southern Queensland and northern New South Wales.

Spore traps. Burkard 'spore and pollen samplers' were sourced from the UK manufacturer; traps incorporate an air intake at 10 l / minute, a revolving internal drum that rotates once every seven days, and an attached sticky tape made from clear plastic coated with petroleum jelly.

Detection of smut spores. Initial diagnosis of spore tapes was by light microscopy. There were several major issues with this method: i. it was very difficult to categorically state that a spore on a spore trap tape was *U. scitaminea* and not that of another smut species, ii. the diagnosis was time consuming, causing there to be significant delays between a spore trapping event and supply of the results, iii. operator fatigue was a very real issue. For this reason, a specific molecular test for *U. scitaminea* was developed and used to assay spore trap tapes. The advantages were the generation of a specific, faster result, though outcomes were then qualitative rather than quantitative (plus or minus smut only)—no quantification of the airborne inoculum was possible using this assay.

Spore trapping program. Fifteen spore traps were purchased soon after the initial smut detection in June 2006; trapping began in the nominated areas in late 2006. Sites were selected across the relevant district with care taken to minimise tape contamination from dust associated with vehicular traffic on unsealed roads and tracks. Traps were operated for 1–2 days at each trap site before movement to another location within the district. Records of weather conditions, site GPS details and crop

details allowed sites to be characterised for later interpretation of results. Mapinfo (version 8) software was used to provide GIS information on smut spore detections.

RESULTS

Early warning. Many detections of sugarcane smut spores within districts and regions were made before disease symptoms were found. In the Burdekin Irrigation Area, over 40% of trap sites returned positive spore trap detections in July 2007. Although careful crop inspections were undertaken at this time, and over the next 18 months, disease symptoms were not detected until October 2008. Similar observations were made in a number of districts (spore detections before crop symptoms); these are listed in Table 1.

Table 1. Spore trap detections of *U. scitaminea* in Australian sugarcane production areas compared to when crop symptoms were first identified.

District	Spores detected	Crop symptoms identified
Mossman	July 2007	December 2008
Tableland	July 2008	September 2008
Mulgrave	July 2008	September 2008
Burdekin	April 2007	October 2008
Proserpine	July 2007	December 2007
Maryborough	March 2007	January 2008

DISCUSSION

The smut spore trapping program successfully provided early warning of the disease in Queensland and New South Wales cane-fields. 18 months pre-emptive warning was provided in the Burdekin and Mossman areas of northern Queensland. Smut spores have been found in northern NSW but after 18 months, no crop symptoms have been seen in this region. Early warning has provided farmers with the opportunity to implement smut management plans much earlier than otherwise would have occurred. As sugarcane is a semi-perennial crop, it is not possible to change cultivars on a whole farm basis within one year; in fact cultivar rotations usually take 4–5 years to complete—so early warning of a disease threat is very important in the transition to resistant varieties. This type of work has not been undertaken in other sugarcane-producing countries. The work reported here illustrates the potential for early warning of sugarcane smut using commercial spore trap technology.

ACKNOWLEDGEMENTS

We acknowledge the assistance of BSES Limited extension staff and Productivity Service personnel.

Software-assisted gap estimation (SAGE) for measuring grapevine leaf canopy density

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INTRODUCTION

Botrytis bunch rot (botrytis), caused by *Botrytis cinerea*, is a major disease of wine grapes. Reducing vine canopy density through leaf plucking can reduce harvest disease severity. Determining the level of leaf plucking required to achieve useful botrytis control requires accurate measurements of canopy density. Existing methods, such as point quadrat (PQ) analysis (1), can be labour-intensive, subjective, damaging, too complicated and/or impractical for many researchers or for routine use by vineyard managers.

Software-assisted gap estimation (SAGE) gives a measurement of the percentage of gap in the vine canopy. This study investigated the relationship between SAGE and PQ analysis, and their relationship to botrytis severity, to establish whether or not SAGE is a satisfactory alternative to PQ analysis for vine canopy density estimation.

MATERIALS AND METHODS

Vineyard Trials. Two trials were conducted during the 2008–2009 growing season on Sauvignon blanc vines in New Zealand, one on a commercial vineyard in Hawke's Bay and the other at the Plant and Food Research vineyard in Pukekohe. Treatments were imposed to produce a range of canopy densities. No botryticides were used in the trials. Canopy density was measured by both SAGE and PQ analysis at pre-bunch closure (PBC), veraison and harvest. An additional measurement was taken at Pukekohe between PBC and veraison.

SAGE. A blue tarpaulin is suspended behind the vine row and is then photographed. The image is analysed using software that calculates the ratio of the area of tarpaulin to the area of leaves in the canopy, termed 'gap'.

PQ Analysis. PQ analysis was carried out as described by Smart & Robinson in *Sunlight into Wine* (1). Leaf layer number (LLN) was compared with gap.

Botrytis severity. Percentage severity of botrytis was visually estimated on 25 randomly selected bunches from each vine.

RESULTS

Gap vs LLN. Gap was found to be highly correlated with LLN (Figure 1). Fitted regression lines for the two sites were not identical. Linear regression analysis testing the hypothesis relating to differences in slope and intercept of the two sites found that the intercepts were significantly different ($P < 0.001$), but the slopes were not significantly different ($P = 0.108$).

Botrytis severity. The mean botrytis severity at harvest in the untreated plots in Hawke's Bay was 6.2% and in Pukekohe was 19.2%. The relationship between canopy density and disease severity at the three growth stages, measured by either SAGE or PQ analysis, was weak but significant in all cases ($P < 0.001$).

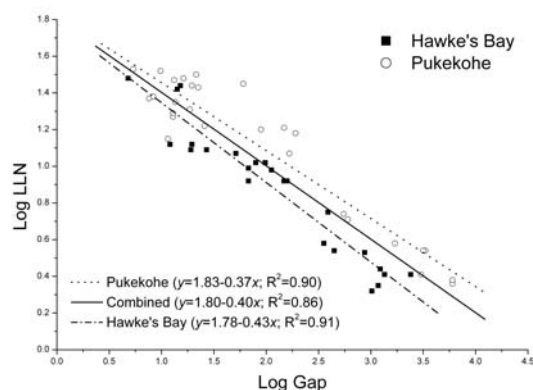


Figure 1. Relationship between gap and leaf layer number (LLN). Regression lines have been fitted to data from Hawke's Bay, Pukekohe and both sites combined. Data shown are log-transformed treatment means for each site at each date.

DISCUSSION

Gap vs LLN. Canopy density measurements from SAGE were highly correlated with those from PQ analysis. The discrepancy in the relationship between sites appeared as an offset, but the proportionality was the same at both sites. To compare gap data with historical LLN data, it would be possible to conduct both SAGE and PQ analysis on ten or more vines and create a standard curve that could be used to predict gap from LLN or *vice versa*. SAGE offers three advantages over PQ analysis: 1) efficiency, 2) reduced subjectivity and 3) an absence of damage to bunches.

Botrytis severity. Higher gap and lower LLN resulted in significantly less disease, demonstrating that leaf plucking had a significant effect on harvest botrytis severity. Further work needs to be conducted into the relationship between gap and botrytis bunch rot severity. Should this relationship be better understood, it lead to better disease control through vine canopy management.

ACKNOWLEDGEMENTS

Funding for this project was provided by the Foundation for Research, Science and Technology (contract C06X0810). We would like to acknowledge David Bishop for his assistance in writing the SAGE software. We would also like to thank Warwick Henshall, Tracy Taylor, Brent Fisher and Dion Mundy (Plant and Food Research, NZ), Kathy Evans (Tasmanian Institute of Agricultural Research, University of Tasmania, TAS, Australia) and Jacky Edwards and David Riches (Department of Primary Industries, VIC, Australia) for their assistance in the developmental stages of SAGE.

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Evaluation of the efficacy of Brassica_{spot}TM models for control of white blister in Chinese cabbage

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INTRODUCTION

White blister caused by *Albugo candida* is a major disease of Chinese cabbage, *Brassica rapa*. *A. candida* causes blisters on abaxial leaf surfaces, necessitating their removal from heads at harvest, which increases production costs. Brassica_{spot}TM, a disease predictive model was developed for white blister on broccoli, *B. oleracea* (1). This abstract reports on evaluation of two versions of the model, the infection model (old) and the latent incubation period model (new) in Chinese cabbage against weekly spray programs for control of white blister.

MATERIALS AND METHODS

Trial design. Chinese cabbage variety Matilda was direct seeded at 3 rows per bed on 27/11/2008 and harvested on 19/1/2009 at a commercial market garden in Devon Meadows, Victoria. The trial was laid out in 6 blocks each divided into 6 plots with unequal replication using the randomised procedure in GenStat. Each plot contained approximately 60 plants. The four treatments applied to plots were: (i) control (unsprayed); (ii) weekly sprays of Tribase Blue (copper sulphate tribase) and Li-700 (soyab phospholipids and propionic acid); (iii) Amistar (azoxystrobin) sprayed according to the old version of the Brassica_{spot}TM model and (iv) Amistar sprayed according to the new version of the Brassica_{spot}TM model. The weekly program received five sprays starting at week 3, the old and new models predicted one spray each at weeks 3 and 4, respectively.

Weather station and Brassica_{spot}TM model. A ModelT weather station (Western Electronic Design) was placed in the crop and recorded average leaf wetness, temperature, relative humidity and total rainfall at 30 min. intervals. The model used this data to predict appearance of symptoms in the crop.

Trial analysis. Disease incidence per plot was recorded as the number of plants out of 20, showing white blister symptoms. A Generalised Linear Mixed Model was used to analyse the data. Severity was scored as the number of the outer 4 free-standing leaves that were showing white blister and these data were analysed using REML (residual maximum likelihood). Due to 100% incidence of white blister on the unsprayed plots, these data were excluded from that analysis.

RESULTS

White blister first appeared in the crop four weeks after sowing in all treatments. At the harvest assessment, leaves appeared to be infected with white blister from oldest to youngest. No white blister symptoms were observed on the leaves covering the head. Conditions favoured white blister consistently throughout the trial, based on the Brassica_{spot}TM model (Fig 1). Amistar applied according to the new Brassica_{spot}TM model was the only treatment to significantly reduce incidence and severity of white blister on Chinese cabbage (Table 1).



Figure 1. Brassica_{spot}TM predictions for the old (top) and new (bottom) versions of the model for prediction for white blister in the Chinese cabbage trial. Old Brassica_{spot}TM model: black bars = high disease pressure, grey bars = moderate disease pressure and white bars = low disease pressure. New Brassica_{spot}TM model: when the 5% line (top) crosses the dashed index bar a spray is predicted (arrow).

Table 1. Effect of timing sprays with the two versions of the Brassica_{spot}TM disease predictive models to control white blister on Chinese cabbage at harvest.

Treatment	Mean incidence on plants (%) ¹	Mean severity on outer 4 leaves (scale 0-4) ¹
Control (unsprayed)	100	2.33a
Weekly	95.59a	2.24a
Old Brassica _{spot} TM model	94.48a	2.17a
New Brassica _{spot} TM model	72.90b	1.17b

¹ Different letters against the predicted means indicates that they are significantly different (p=0.008)

DISCUSSION

Disease freedom on the outer 4 leaves of the harvested head is a critical commercial quality factor, but none of the treatments achieved this. Amistar applied according to the new Brassica_{spot}TM model was the only treatment giving significant control of white blister on these outer leaves. The new model recommended a single spray 14 days before harvest, based on disease progression data from the crop inspections and environmental data, but spraying 14 d prior to harvest may co-incidentally be the best phenological timing to protect the 4 unfolding leaves. Using the model is time consuming because it involves crop inspections. Further work is suggested to compare spraying according to new Brassica_{spot}TM model against a single spray of fungicide 14 d before harvest.

ACKNOWLEDGEMENTS

The authors thank HAL, AusVeg, the State Government of Victoria and the Federal Government for financial support and the growers for providing field trial sites.

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evaluating an infection model of prune rust to improve the management of disease for almond and prune growers

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INTRODUCTION

Trials of an almond rust infection model could lead to financial savings by effectively controlling the disease with minimum chemical use. The threat of infection events for almond and prune rust (*Tranzschelia discolor*) in orchards is responsible for many fungicides being applied needlessly. We aim to identify the conditions that favour the pathogen to 1) more effectively predict disease and thus more accurately time spray applications, and 2) address key issues such as: the rising cost of fungicides and fuel, and the industry's move to improve the carbon footprint of almond orchards. We plan to assess the potential for a disease advisory service for almond and prune growers similar to CropWatch as used in the South Australian grape industry.

MATERIALS AND METHODS

For the past two years, *Model T MetStations*[®]—two at Loxton in the Riverland and one on the Adelaide Plains—have monitored orchard micro-climate while we have monitored disease progress. An infection model developed for prune rust (1) has been adapted for use in almonds and installed in the *MetStations*[®] as disease predictors. The *MetStation*[®] software used the model to process leaf wetness and temperature data and predict infections. We compared these with field observations to evaluate the model for accuracy. The foliage was monitored usually every 8 days (range 2–13 days).

RESULTS

We present data for 2008/09 at Loxton. In that season, rain events of >1.5 mm were rare: x2 in September; x1 October; x3 November; x4 December and none in January-March. Consistent with the dry conditions, new occurrence of rust (light infection only) occurred on only four occasions (Table 1).

For example, on 2 November, a 10.7 mm rain induced 15 hours leaf wetness while temperatures ranged from 24.8°C–13.8°C. The model predicted an Infection Score (InfSc) of 5026 for this event. Since this was below the previously set threshold of 6,500 for significant disease (data not shown), a light infection was expected. Previous experiments had measured incubation periods of between 17–21 days, so we anticipated a little rust would be first seen in the vicinity of 19 November. This matched well with observations of a few rust pustules on 17 November which increased in number by 24th.

Table 1 shows the model outputs for this and three other events in which infection was observed. Similar or better accuracy was

achieved on each of those occasions but an apparent failure occurred on two others, when no rust developed.

In the period January-March 2009, there were many days with no leaf wetness. The prototype model correctly predicted 'no disease' for these events.

DISCUSSION

The rarity of the rain events made it possible to decipher when infection actually occurred. One of the 'failures' occurred on 28 November with a 12-hour leaf wetness period for which the model predicted InfSc 3920, a light infection, comparable to that noted on 12 December, but none was seen. Analyses of the data for this and an additional predicted light infection event on 5 December showed that relative humidity (RH) was low for much of the associated leaf wetness periods. This raised the question: would the prototype infection model be improved by adding a RH factor?

Further review of the model is planned in subsequent seasons but evaluation to-date suggests incorporation of RH as a factor would increase the model's sensitivity in distinguishing potential light infections caused by leaf wetness alone, from conditions when RH is also high and more favourable for infection. For instance, a simple arbitrary multiplier could be included as follows:

RH 00.0 – 89.9%,	InfSc = InfSc x 1.0 (ie no change)
RH 90.0 – 97.9%,	InfSc = InfSc x 1.2; and
RH ≥ 98%,	InfSc = InfSc x 1.5.

Conclusions The potential for success in adapting the *Model T MetStation* as a rust disease predictor to-date has led to optimism in achieving project objectives. The current data have advanced the prospects of the prototype infection model as a useful tool for almond (and prune) growers to manage disease. The model has shown capacity to provide advice as to when sprays can be confidently withheld and when they are needed in rust control programs.

ACKNOWLEDGEMENTS

We appreciate the assistance of Ben Brown, the Almond Board of Australia, and Horticulture Australian Ltd in facilitating this project.

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Table 1. Predictions of Infection Events and Disease Scores Compared to Observations of the Almond Rust Fungus *Tranzschelia discolor*. Site 1: Loxton Research Centre, Loxton, SA. 2008/09

Date	Time	Score, Severity and Date of Predicted Disease			Orchard Disease Observations
2 Nov	16:00	5026	Light infection	From 19 Nov	Between 17–24 Nov. Few pustules
13 Nov	23:40	5338	Light infection	From 30 Nov	Between 26 Nov – 2 Dec. Few pustules
28 Nov	01:30	3920	Light infection	From 15 Dec	None seen
5 Dec	02:50	3528	Light infection	From 22 Dec	None seen
12 Dec	12:30	3808	V. light infection	From 29 Dec	On 29 Dec. Few pustules
18 Dec	01:30	4088	Light infection	From 4 Jan	Between 29 Dec and 6 Jan. Few pustules

Management of white blister on vegetable brassicas with irrigation and varieties

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INTRODUCTION

White blister caused by the oomycete *Albugo candida*, has been the main foliage disease on red radish for at least 30 years. In the summer of 2001/2002, it caused up to 100% crop losses in broccoli and cauliflower in Victoria. *A. candida* infecting radish is generally classified as race 1 and *A. candida* infecting broccoli is classified as race 9.

This abstract reports on systematic surveys undertaken seasonally during 2002 to identify if irrigation practices impacted upon the level of disease in commercial red radish crops, and whether similar IPM practices could be used to manage white blister on broccoli crops.

MATERIALS AND METHODS

Systematic surveys. Red radishes of all ages were assessed seasonally during 2002 by a 'two-stage sampling method' (Nam Ky Nguyen, pers. comm.). This method was used to determine the number of beds to be selected for assessment of white blister in each half of a bay (section between over-head sprinkler lines). The beds were then randomly selected. A bed of radish usually consists of 6 rows. In each of the selected beds, two sections each of 20 cm in length were randomly selected to assess for the incidence of white blister. During summer, autumn, winter and spring 26, 23, 25 and 27 crops were surveyed, respectively. Only growers who irrigated crops at the same times were included in the analysis. Due to the binary nature of the data, logistic regression was used to analyse the results.

Irrigation field trial for broccoli. The trial, located at Dairy Road, Werribee, Victoria, was originally designed as a general split-plot design. Each of 3 blocks contained 2 replicates. Each of the 6 replicates consisted of two whole plots to which the two irrigation times of early morning (4.00 am) or evening (8.00 pm) were randomly allocated. The 8 treatment combinations of variety (consisting of the resistant variety 'Tyson' from Syngenta, or the susceptible variety 'Ironman' from Seminis) and four spray regimes (not reported here) were randomly allocated to 8 subplots within each of the whole plots.

Seedlings, aged 8 weeks, were planted 2 rows per bed on 23 July 2008. Subplot dimensions of beds were 8 m long and contained approximately 52 plants. The middle 20 plants per subplot were assessed for the incidence (presence or absence) of white blister on broccoli heads at harvest. A generalised linear mixed model (GLMM) was fitted to the data.

RESULTS

Systematic surveys of red radish. White blister on red radish was most prevalent during autumn and spring (Table 1). Red radish crops irrigated in the evening (8.00pm–12.00pm) had significantly higher incidence of white blister compared with other times of irrigation. Crops that were irrigated in the early morning (approximately 6.00 am) showed lower levels of white blister in 3 of the 4 seasons surveyed (Table 1). Data collected on age of crop, cultivar and regional differences are not reported here.

Table 1. Effect of irrigation time on the incidence of white blister on red radish

Time of irrigation	Incidence of white blister in 2002 (%)			
	Summer (Feb and Mar)	Autumn (May)	Winter (Jul and Aug)	Spring (Oct and Nov)
Evening (8-12pm)	7.9a ¹	23.0a	4.8a	12.5a
Late morning (9-12am)	0.8b	12.1b	0.4c	4.9b
Early morning (approx.6.00)	0.8b	3.4c	1.7b	1.5c

¹Within each column (season), different letters against the means indicates that they are significantly different at the 5% level.

Irrigation trial for broccoli. Irrigating the broccoli crop in the evening approximately doubled the incidence of white blister compared with early morning irrigation for both varieties (Table 2). Tyson which is a resistant variety showed few symptoms of white blister.

Table 2. Effect of irrigation time on broccoli varieties susceptible and tolerant to white blister

Time of irrigation	Mean incidence (%) of white blister	Mean incidence (%) of white blister	
		var. Tyson	var. Ironman
Evening (8.00 pm)	2.9 a	0.57	14.37
Early morning (4.00 am)	1.3 b	0.25	6.8

The means are significantly different ($p=0.005$).

DISCUSSION

Early morning irrigation (4.00 am or 6.00 am) can be a useful IPM tool to reduce the risk of white blister in red radish and broccoli crops. Growing a resistant variety may further reduce the risk of white blister.

ACKNOWLEDGEMENTS

The authors thank HAL, AusVeg, the State Government of Victoria and the Federal Government for financial support and the growers for providing field trial sites.

Alternative screening methods for sugarcane smut using natural infection and tissue staining

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INTRODUCTION

Sugarcane smut caused by *Ustilago scitaminea* was first detected in the Ord River Irrigation Area, WA in 1998, and in Queensland in 2006 (1). BSES had rated cultivars for resistance to smut in Indonesia and WA prior to the arrival of the disease in Queensland but commenced a program to screen sugarcane clones for resistance in Queensland when it clear the disease could not be eradicated. The current screening method involves inoculation of sugarcane setts by dipping into smut spore suspension. Although this method is internationally accepted, it has some drawbacks: i) it does not replicate natural infection; and ii) it is relatively time consuming and expensive.

The objectives of this research were: i) obtain data on sugarcane cultivar reaction to natural infection and compare with existing standard ratings; and ii) develop a histological method to screen for smut resistance.

MATERIALS AND METHODS

Natural infection. Nine cultivars with a range of smut ratings were planted at the Bundaberg smut research farm, using randomised complete block design with 10 replicates in September 2007. Rows of smut susceptible cultivar Q205^A inoculated with smut were planted between the rows of the test plots. The trial was inspected for smut twice in 2008 in the plant crop, and monthly from February 2009 in the first ratoon (re-growth) crop. The number of infected plants and total number plants in each plot were counted. Data were log-transformed ($\log_{10}(\text{smut}\%+1)$) for regression analysis to determine the relationship between standard smut rating and smut incidence for natural infection.

Tissue staining. Eight cultivars of various smut ratings were cut into one-eye-setts, inoculated in smut spore suspension, and planted in pots. The pots were transplanted into the field after 10 weeks. The plants are being assessed at 0, 1, 2, 4, 6, 8, 10 weeks in the glasshouse, and 26 weeks in field. For assessment, >1 mm sections of bud were cut, and stained with trypan blue (Figure 2), and observed under a light microscope, and subsequently photographed.

RESULTS AND DISCUSSION

Natural infection. Except Q205^A, no smut symptoms were observed in the plant crop in 2008. High incidence of smut was observed on susceptible cultivars (rating 6–9) in the first ratoon crop compared with resistant cultivars (Table 1). The regression results suggest that there was a highly significant ($P<0.001$) correlation between standard ratings and smut incidence in natural infection trial. The main drawback of the natural infection method is it takes two to three years to get results, whereas the standard smut testing method takes only eight to 10 months.

Tissue staining. Fungal colonisation was observed two weeks after inoculation in 4 of the 5 susceptible and intermediate cultivars. In resistant cultivars, only, 1 cultivar (Q232^A) out of 3 showed evidence of colonisation (Figure 3). Although the experiment is in a preliminary stage, this result suggests that

there is potential to reduce the time of screening by discarding susceptible cultivars early in the screening program.

Table 1. Incidence (%) of smut infected plants in natural infection trial in Bundaberg, assessed in March 2009

Cultivar	Standard rating	Mean smut % *
Q151	1	1.43 (1.43)
Q232 ^A	3	2.00 (2.0)
Q190 ^A	4	9.71 (5.5)
QS97-2067	4	6.43 (5.0)
Q135	5	12.20 (4.6)
QS94-91	6	23.02 (6.5)
Q188 ^A	7	22.08 (5.6)
Q138	8	21.78 (3.3)
Q205 ^A	9	93.17 (3.3)

* values in parenthesis are \pm standard error of means

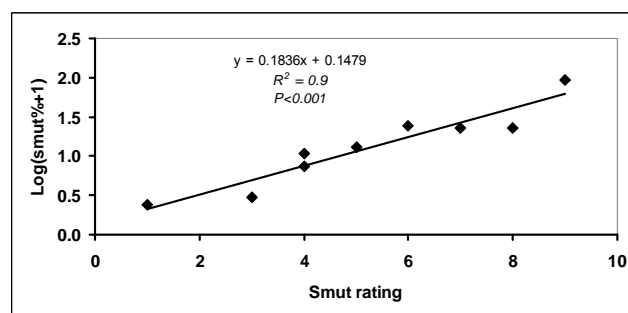


Figure 1. Relationship between standard smut rating and incidence of smut in natural infection experiment in Bundaberg



Figure 2. Procedures of sectioning and staining of sugarcane bud tissue

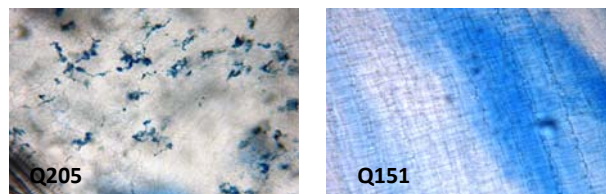


Figure 3. Smut colonisation in tissue of smut susceptible cultivar (Q205^A) compare to smut resistant cultivar (Q151)

ACKNOWLEDGEMENTS

Sugarcane Research and Development Corporation (SRDC) funded this research work.

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Interruption of cool chain and strawberry fruit rot by leak-causing fungi *Rhizopus* species

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INTRODUCTION

The causal agents of strawberry leak are several different species grouped in the fungus-like class of zygomycetes (kingdom: Chromista), which are fast growing organisms¹. Zygomycetes have been associated with strawberry fruit leak, mostly from the *Rhizopus* genus, but similar disease symptoms are also associated with *Mucor* infections¹. The recent detection of several cold-tolerant leak isolates² has prompted research into cool chain management for NZ strawberry. The work described here shows how breaks in the cool chain increase leak rots and therefore dramatically decrease the shelf-life of strawberry fruit.

MATERIALS AND METHODS

Six leak isolates (*Rhizopus stolonifer*) were used to inoculate (mycelium) potato dextrose agar (PDA) and sliced strawberry fruit (5 mm). Cool chain incubation (4°C) was interrupted at staggered intervals (daily) by exposure to room temperature (20°C) for 2 h, resulting in continuous cool chain incubation or 1–6 interruptions. Fungal growth was assessed daily during the '2 h at room temperature cycle'. There were 2 replicates/isolate and substrate. The experiment was repeated.

An additional whole fruit experiment was conducted. Eight fruit per tray (with 10 individual compartments) were inoculated with a mycelial tuft from one of the six isolates and two fruit served as non-inoculated controls (injury only). There were two replicate trays per isolate. Incubation and interruptions were as above. Rot was assessed employing a fruit score, where 0=no symptoms; 1=small sunken lesion; 2=large sunken lesion; 3=sunken lesion with juice leaking; 4=fruit covered in mycelia. At completion of the experiment all fruit were left for 2 days at 20°C to check for delayed onset of disease symptoms.

RESULTS

Mycelial growth and fruit score for continuous incubation at 20°C and 4°C (with 1–6 interruptions) are shown in Figures 1. Growth and disease symptoms increased during incubation at both temperature regimes. At the 4°C incubation, the number of interruptions (or hours exposed to 20°C) significantly increased ($P<0.001$) mycelial diameters and leak score (Figure 2). At 4°C, final growth or disease score was very similar for a single interruption event irrespective of whether interruption occurred on day 1 or day 6 of the experiment.

DISCUSSION

The work confirms that cold-tolerant strains of *Rhizopus* isolates exist². We demonstrate that under incubation at 4°C, leak rots occur when fruit are inoculated with mycelium. This is in contrast to conventional literature reporting control of *R. stolonifer* disease development in strawberry at temperatures below 6°C¹. In our work, while incubation at 4°C slowed growth and disease development, once the cold chain was disrupted (even for as little as 2 h) growth and disease progressed at a faster rate, clearly reducing fruit shelf-life. Therefore it is important that fruit are cooled as quickly as possible after harvest and that the cool chain is not interrupted throughout the supply chain.

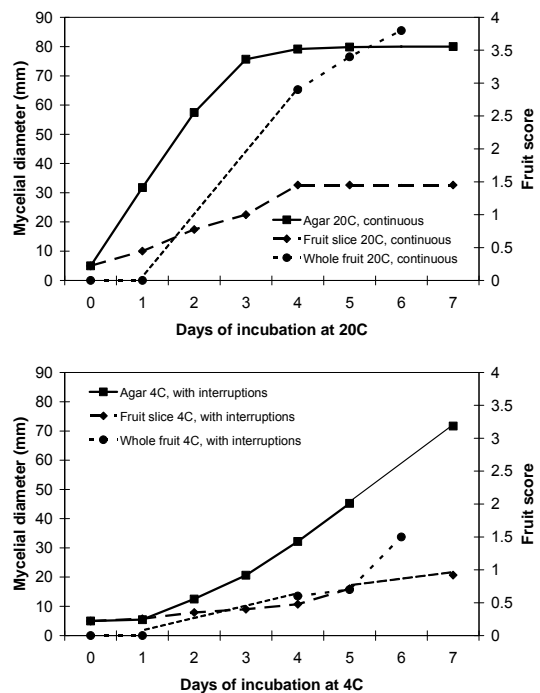


Figure 1. Mycelial diameter and disease score of *Rhizopus stolonifer* isolates on PDA (SE=3.1), strawberry fruit slices (SE=1.7) and whole fruit (SE=0.13) during 7 days of continuous incubation at 20°C (top) and 4°C (bottom).

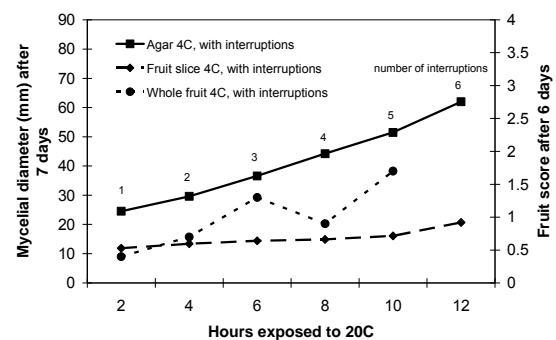


Figure 2. Mycelial diameter and disease score after 7 and 6 days of incubation for of *Rhizopus stolonifer* isolates on PDA (SE=3.2), strawberry fruit slices (SE=1.8) and whole fruit (SE=0.12) incubated at 4°C with 1–6 interruptions.

ACKNOWLEDGEMENTS

The work was funded by Strawberry Growers New Zealand Inc. and the Sustainable Farming Fund (SFF) of the Ministry of Agriculture and Forestry (MAF) Grant L08/079.

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Enhancing Papua New Guinea smallholder cocoa production through greater adoption of integrated pest and disease management

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INTRODUCTION

More than 80% of Papua New Guinea's annual cocoa production is produced by 150,000 smallholder farming families. The current average yield of 300 kg dry beans/ha reflects poor management and high losses to *Phytophthora* pod rot and canker (*Phytophthora palmivora*) and Vascular Streak Dieback (*Oncobasidium theobromae*). Since 2007 cocoa pod borer (CPB) has also been recognised as a serious pest after the eradication program in Gazelle district of ENB failed. Apart from improved cocoa genotypes, technology adoption is poor and over 95% of 108 farmers surveyed had no knowledge of cocoa disease and pest management, leading observers like Frank Jarrett (1) to ask "how do farmers (in PNG) find out about innovations and just what sources of information are important?"

We developed interventions that are synchronised with the cocoa cropping cycle and the resources available to farmers and link stakeholders through active participation. Four Integrated Pest and Disease Management (IPDM) options have been piloted in 3 different provinces.

MATERIALS AND METHODS

The IPDM strategy was designed with inputs programmed in relation to peak flowering, cherrille setting and peak ripening and also in relation to the pest and disease cycle so that the IPDM inputs are applied when the pest and disease are at their weakest point of their cycle (2). Four IPDM packages including a conventional management option were tested in East New Britain (ENB), Bougainville and Madang (Table 1).

Table 1. IPDM options developed for PNG cocoa farmers

Option	IPDM Input	Activities
1	Low	Current practice (minimal)
2	Medium	Sanitation, weekly harvests, cocoa and shade tree pruning, weed management
3	High	Option 2 + canker treatment, fertiliser and manures
4	Very high	Option 3 + insect management

The piloting of options involved three village communities in Bougainville, Madang and ENB. At each site 12 to 15 farmer families were selected and the four options were established through Participatory Action Research (PAR). In the start up workshop in November 2005, selected and interested farmers participated in presentations and discussions. Some smallholder communities were provided with information on improved cocoa management from trials at the CCI research station. Selected farmers, their families and extension officers were trained and were engaged in observing and analysing the condition of trees in each option before applying treatments for each management package. Each selected family treated one tree in each option and the trees were given the name of the farmer involved. The four options were applied in each farmers block, and fully replicated by the 12 participating farmers. Thus there were 36 farmers trained in each province. Farmers visited their trees every month, carried out observations and took

notes. The managed cocoa tree themselves have become the farmer's principle educator. Baseline surveys were carried out at the beginning and a second followup survey using the same questionnaire was conducted in 2008 to determine if farmers had changed their management of cocoa.

RESULTS AND DISCUSSION

We aim to transform the industry from the current 90% low input to 50% medium input farms. Over 108 PAR trials have been established and more than 1500 farmers trained. The uptake of options is close to 100%, and over 80% of farmers prefer the higher input options. Yield increases of more than 100% have been reported and PNG smallholders are investing in cocoa for the first time. National production has increased from 42,000T in 2005 to 56,000T in 2008. Farmers in CPB-infested areas in ENBP report increased yields following the implementation of IPDM despite the impact of CPB.

In the delivery of IPDM, direct transfer of the technology and establishment of research programs with farmers via PAR provides a unique opportunity to increase adoption of research results. Through this approach smallholders were trained to record inputs and production data, and are able to understand plant health management and develop improved cocoa management and production.

Establishing demonstration plots and conducting field days has increased the profile of research and extension agencies, which are now much more engaged with the day-to-day problems faced by the farmers. The feedback from farmers has in turn improved the capacity of supporting researchers at CCI to focus their research on industry needs.

The work highlights the importance of packaging research results into recommendations to improve technology adoption.

ACKNOWLEDGMENTS

This work was supported by ACIAR (PHT/2003/015).

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Molecular cytology of *Phytophthora*-plant interactions

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INTRODUCTION

Many of the more than 60 species of *Phytophthora* are aggressive plant pathogens that cause extensive losses in agricultural crops, horticultural plants and natural ecosystems. Some *Phytophthora* species have narrow host ranges; others have extremely broad host ranges. *P. cinnamomi*, for example, is now known to infect over 3,500 plant species, many of them native to Australia.

Phytophthora and other members of the class Oomycetes form fungus-like hyphae and conidia-like asexual sporangia, but they are not fungi. The Oomycetes group with a range of other protists such as diatoms, coloured algae and malarial parasites within the Stramenopiles, an assemblage whose taxon-defining characteristics include possession of tubular hairs on their flagella.

Species of *Phytophthora* produce motile, biflagellate zoospores that play a key role in the initiation of plant disease. Zoospores target suitable infection sites where they encyst and attach. Cysts soon germinate and attempt to invade the underlying plant tissues. Some *Phytophthora* species are hemibiotrophs and initially establish a stable relationship with living host cells, obtaining nutrients through the development of haustoria within infected cells. The majority are necrotrophs that feed on dead or dying cells. Like fungi, *Phytophthora* and other Oomycetes secrete effector proteins that are required for pathogenicity. Some effectors, such as cell wall degrading enzymes, function in the plant apoplast but others are transported across the plant plasma membrane into the host cytoplasm from where, in susceptible plants, they orchestrate metabolic changes that favour pathogen growth. In resistant plants, recognition of the invading pathogen induces a rapid defence response that inhibits disease development. In this presentation, I will review our current understanding of cellular and molecular aspects of the interactions between plants and *Phytophthora* pathogens. In so doing, I will highlight how modern molecular cytology is revolutionising our ability to elucidate the roles of selected proteins and cell components in *Phytophthora* pathogenicity and plant defence.

MATERIALS AND METHODS

Early studies of the interactions between plants and species of *Phytophthora* used light and electron microscopy to describe the major features of disease development and the plant defence response. More recently, these traditional approaches have been extended by advanced light and electron microscopy techniques that use a variety of methods, such as immunocytochemical labelling, GFP-tagging and confocal microscopy, to mark and visualise a range of plant and pathogen molecules and cell components. This molecular cytology not only facilitates identification of specific cell structures in fixed and sectioned material but it can also do so in living cells.

RESULTS AND DISCUSSION

***Phytophthora* pathogenicity.** *Phytophthora* zoospores are chemotactically and electrostatically attracted to specific regions on the plant surface that are favourable infection sites. Zoospores encyst and attach to the plant through rapid secretion of adhesive material that includes high molecular weight proteins containing multiple thrombo-spondin type1 repeat

motifs. Cysts germinate from a pre-determined site that has been oriented towards the plant surface. Germ tubes penetrate either directly through the outer periclinal wall or along an anticlinal wall. *Phytophthora* species contain large multigene families encoding cell wall degrading enzymes whose secretion facilitates penetration and colonisation of host tissues. Nutrients are acquired from living or dead plant cells, allowing host colonisation and pathogen reproduction within 2–3 days.

Plant defence. Plants react rapidly to attempted infection by *Phytophthora*. Some of the earliest responses observed include an increase in cytoplasmic Ca^{2+} concentration, cytoplasmic aggregation, formation of wall appositions beneath the invading hyphae and synthesis of reactive oxygen species, pathogenesis-related proteins and phytoalexins. Immunocytochemistry and GFP-tagging have revealed that cytoplasmic aggregation is accompanied by dramatic and dynamic reorganisation of actin and microtubular cytoskeletons, the endoplasmic reticulum (ER), Golgi bodies (GA) and peroxisomes. Actin, ER, GA and peroxisomes become focused on the infection site and are likely to be responsible for secretion of toxins and formation of wall appositions that inhibit hyphal penetration of the plant cell wall. Recent studies of GFP-tagged *Arabidopsis* plants indicate that the rapid plant cell response may be triggered by detection of the pressure exerted by the invading pathogen hypha.

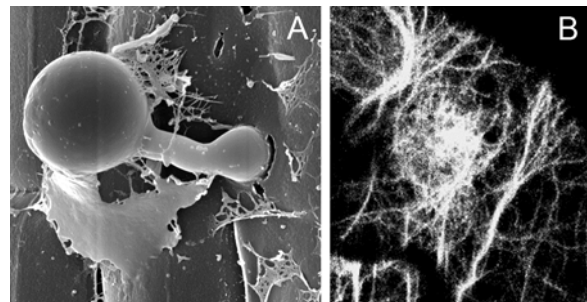


Figure 1. Attack and defence. **A.** CryoScanning electron micrograph of a *Phytophthora* spore that has penetrated the plant surface along the anticlinal wall between adjacent epidermal cells. Events leading up to this stage include zoospore chemotaxis to a suitable infection site, polarised cyst germination and secretion of cell wall degrading enzymes from the hyphal tip. **B.** Confocal microscopy of transgenic *Arabidopsis* plants expressing GFP-tagged hTalin to visualise actin microfilament arrays in living plant epidermal cells responding to attack. Aggregations of filamentous actin form directly underneath sites of attempted penetration.

The genomes of four *Phytophthora* species have now been sequenced with others in the pipeline. Extensive transcriptome data are also available for *P. infestans* and *P. nicotianae*. Together with data from a number of host plants, this sequence information provides an invaluable resource for molecular cytology studies of protein and organelle function during *Phytophthora*-plant interactions.

Gene expression changes during host-pathogen interaction between *Arabidopsis thaliana* and *Plasmodiophora brassicae*

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INTRODUCTION

Plasmodiophora brassicae is a biotrophic obligate plant pathogen that causes developmental changes in susceptible host cells, leading to the development of root galls (clubroot) and stunted growth. It is one of the most devastating diseases of vegetable brassicas worldwide and significantly reduces crop yield. In Australia, clubroot is managed using a combination of integrated control methods and recently introduced resistant varieties. Breeding resistant cultivars is difficult because of the genetic variation in the field populations of the pathogen. A host-pathogen interaction between *Arabidopsis thaliana* ecotype Col-0 and *Plasmodiophora brassicae* (Australian field population) was examined at the cellular and molecular levels to gain a better understanding of the pathogenic mechanisms. This study reports on the gene expression study (microarray analysis) conducted for this compatible host-pathogen interaction during the key developmental stages of the disease prior to ten days after inoculation.

MATERIALS AND METHODS

A modified sand-liquid culture method was developed to grow test-plants such that observation of the primary life-cycle stages of *P. brassicae* within *Arabidopsis* (ecotype Col-0) roots was possible at very early time points. A real-time quantitative PCR (qPCR) assay was also developed to quantify *P. brassicae* DNA in roots from day 1 onwards and up to 23 days after inoculation. Microarray analysis was conducted at 4, 7 and 10 days after inoculation using the 22K *Arabidopsis* ATH1 microarray chip. Gene expression at greater than a 1.5-fold increase or decrease at a 95% confidence level relative to controls was considered biologically significant in this study. Data analysis was carried out using the AVADIS software package and selected genes were validated using real-time reverse transcriptase quantitative PCR (RT-qPCR).

RESULTS AND DISCUSSION

According to the microscopic study, pathogen attachment and penetration occurred from day 4 onwards and root galls were fully developed within 28 days. QPCR confirmed that *P. brassicae* DNA was detectable in infected *Arabidopsis* roots from day 4 onwards. The amount of amplified pathogen DNA increased by day 23 as the disease progressed equating to the amount of pathogen DNA amplified from 10⁸ resting spores.

Microarray analysis conducted at these early time points (days 4, 7 and 10) demonstrated significant changes in gene expression. At 4 days after inoculation (dai), 147 genes were differentially up- or down-regulated relative to control plants compared to 27 genes at 7 dai and 37 genes at 10 dai. All genes were categorised into their functional groups and metabolic pathways. At day 4, when the pathogen had attached to the root hair and had possibly commenced penetration, differential expression of several genes known to be important for pathogen recognition and signal transduction in resistant interactions, such as the WRKY transcription factor, TIR-NBS-LRR and leucine-rich repeat protein, were induced. Genes involved in cell growth, jasmonic

acid biosynthesis and lipid biosynthesis were also induced. However, genes involved in the biosynthesis of lignin, phenylpropanoids (salicylic acid), ethylene, cytokinin, reactive oxygen species and pathogenesis related proteins (chitinase) were repressed (Table 1).

Table 1. Summary of up- and down-regulated genes expressed in *Arabidopsis* roots 4 days after inoculation with *P. brassicae*

Up-regulated genes	Down-regulated genes
Signalling	Oxidative burst/stress
WRKY transcription factor	peroxidase
leucine-rich repeat protein	glutathione S-transferase
MAPKK	NADP oxidoreductase
CDPK	Lignin biosynthesis
TIR-NBS-LRR	Salicylic acid biosynthesis
Cell growth and modification	Ethylene biosynthesis
expansin	Cytokinin biosynthesis
xyloglucan:xyloglucosyl transferase	PR proteins
pectinesterase	
hydroxy proline-rich glycoprotein	
arabinogalactan	
Jasmonic acid biosynthesis	
Lipid metabolism	

Genes differentially expressed were fewer in number at the 7 and 10 day time points, which is the time when the pathogen has established within the roots and has developed into primary plasmodia and zoosporangia, without any morphological changes in the host. Four candidate genes expressed at day 4 (WRKY transcription factor, lipoxygenase, phytoalexin-deficient 4 protein and TIR-NBS-LRR) were confirmed by RT-qPCR.

In conclusion, the microarray study identified changes in gene expression among many host-plant genes that are known to have important roles during plant-pathogen interactions that may be amenable to manipulation to increase disease resistance. Microscopic observations of host roots during the infection process showed correlations between gene expression and pathogen life-cycle stage. The most important time point, in terms of gene-expression changes in the plant was 4 days after inoculation. Suppression of specific gene activity and/or functional groups of genes in the host may lead to susceptibility in this host-pathogen interaction.

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 would also like to thank DPI, Victoria for providing access to facilities.

Hairpin RNA derived from viral NIa gene confers immunity to wheat streak mosaic virus infection in transgenic wheat plants

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INTRODUCTION

Wheat streak mosaic virus (WSMV) has recently been identified from wheat and other cereals in Australia. The difficulties in finding adequate natural resistance in bread wheat and durum wheat prompted us to develop transgenic resistance based on induced siRNA mechanisms. We are reporting a successful strategy to develop resistance in Wheat against WSMV.

MATERIALS AND METHODS

A hairpinRNA construct was designed derived from Nuclear Inclusion ‘a’ gene of WSMV. BobWhite26 was stably co-transformed with two separate plasmids: one containing a hairpin with WSMV sequences and the other one with the *nptII* selectable marker.

RESULTS

Using biolistics we obtained a transformation efficiency of 3.5%. When progeny T₁ individuals were assayed against WSMV ten out of 16 tested families showed extreme resistance in transgenic segregants. The resistance in transgenic T₁ segregants was classified as immunity by four criteria: no disease symptoms were produced; ELISA readings were as in uninoculated plants; viral sequence could not be amplified from sap; the same saps failed to give infections in susceptible plants when used in test-inoculation experiments. In one of four transgenic families examined in greater detail, the resistance segregated in a simple Mendelian ratio along with the transgene (Fig 1); the T₀ parent (hpWS2b) of this family had a single transgene insert by Southern hybridisation. Also in the T₁ family of hpWS2b the antibiotic resistance gene *nptII*, introduced on a separate plasmid by co-bombardment, segregated independently of the hairpin transgene.

DISCUSSION

This paper reports for the first time engineered RNAi mediated immunity in wheat against WSMV using a hairpin RNA derived from Nuclear inclusion protein a (NIa) protease gene. WSMV is arguably the third most important virus of wheat behind BYDV and CYDV (barley and cereal yellow dwarf viruses). Marker-free transgenics have advantages for regulatory approval and public acceptance (1, 2). Our study indicates that marker-free WSMV immune plants can be readily produced using hairpinRNA genes, biolistics and co-bombardment.

ACKNOWLEDGEMENTS

We acknowledge AusAID for the studentship support of MF, Peter Waterhouse for invaluable advice on hairpin RNA design, Terese Richardson and Anna Mechanicos for technical support.

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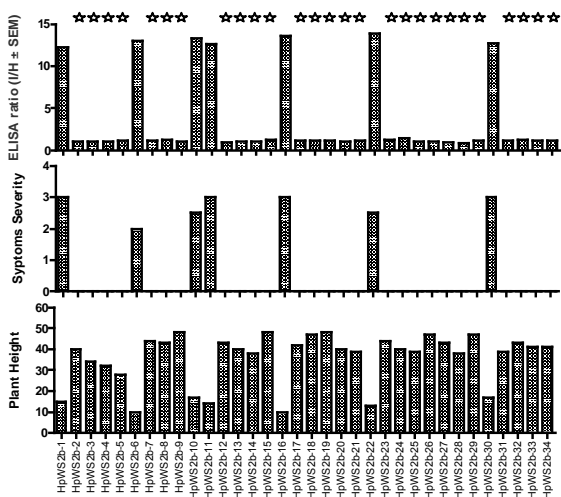


Figure 1. WSMV inoculation of hpWS2b T₁ transgenic family. Shown are the ELISA ratio at 14 dpi, symptom severity and plant height at booting stage. The asterisk shows which plants amplified both ends of the hairpin transgene. This family showed simple mendelian inheritance of the transgene cosegregating with the immunity.

Characterising inositol signalling pathways in *Phytophthora spp.* for future development of selective antibiotics

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Phytophthora is probably best known for causing the Irish potato famine of the 1840s, but this plant pathogen is not just an issue of history. Recent estimates show *P. sojae* to cause \$1–2 billion in soy bean and \$400 million in tomato crop losses p.a., and that *P. infestans* costs ~\$290 million p.a. in management and losses of potato crops in the USA (e.g. 1). In Australia, *P. cinnamomi* is one of the greatest risks to our terrestrial ecosystems: it destroys numerous non-arid habitats, having a wide host range of up to 2500 plant species (2), and is thus considered a key threat under the Commonwealth Environmental Protection and Biodiversity Conservation Act 1999. Development of a control for *Phytophthora spp.* is critical to future sustainable agriculture and will be invaluable in maintaining numerous ecosystems in Australia and abroad. This project aims to directly target *Phytophthora* in the same manner used to develop the anti-influenza drug, Relenza (3) by identifying and solving the structure of a protein(s) unique to and critical for the survival of *Phytophthora*, we aim to provide the information required for future development of customised antibiotics.

Only a handful of biological components are so critical to life that they are conserved throughout all organisms. For example, ribosomes are required for protein synthesis and as such are found in every living cell. So too, several signaling cascade enzymes appear to have been conserved across life; phospholipase C (PLC) is one such enzyme conserved from bacteria to humans and, just as the loss of ribosomes would be fatal, the loss of PLC would be catastrophic to the cell. How is it then, that plant pathogens of the genus *Phytophthora* do not have any recognizable PLC (4)? Phospholipase C is a transient membrane protein that, upon GTP activation, hydrolyses the phospholipid phosphoinositide bisphosphate (PIP₂) into the secondary messengers (1,4,5) inositol triphosphate (IP₃) and diacylglycerol (DAG), which *inter alia* activate protein kinase pathways, phospholipase D pathways and mediate rapid calcium release from the endoplasmic reticulum (5).

We propose that PLC has been replaced by an alternative protein we call AltPLC. Existence of such an alternate protein would not only represent significant insight into the evolution of *Phytophthora*, but may indeed represent an ideal target for anti-*Phytophthora* antibiotics.

We have approached the problem of identifying the AltPLC from three directions, utilising structural bioinformatics, differential proteomics, and biochemical analysis.

METHODS

Structural bioinformatics: We have identified all proteins within the *P. sojae* genome which bind to PIP₂ using Hidden Markov Models to search for patterns which convey the structure of Plekstrin homology domains—a structure known to specifically bind to PIP₂. This data set was then scrutinised by a number of domain-architecture mapping and structural prediction algorithms.

Differential proteomics: we have developed a method of isolating transient membrane proteins. This involves cracking the cells under high calcium and low temperature conditions, to bind

lipid-regulating proteins to the membrane fragment. After washing the membrane, elution is achieved by removing Ca²⁺ and allowing dissociation. Analysis of these fractions was performed by MS/MS and *in vitro* hydrolysis reactions.

Biochemical analysis: IP₃ was isolated using the method of Lorke *et al.* (2004)(6) and analyzed by MDD-HPLC (7).

RESULTS

Using MDD-HPLC we have shown that *P. cinnamomi* does produce IP₃ endogenously and DAG *in vitro* by hydrolysis reactions with differentially isolated transient membrane protein fractions. This evidence supports our hypothesis of an alternative PLC in the *Phytophthora* genus. Using our combinatorial bioinformatics approach we have uncovered a single protein with all necessary structural components to perform PIP₂ hydrolysis. Furthermore, this protein is conserved among *P. sojae*, *P. ramorum* and *P. infestans* and, as hypothesised, is unique to the *Phytophthora* genus. We have cloned and continue to isolate, recombinant AltPLC and its activator RAS protein for functional and structural analysis. although final correlation between PIP₂ hydrolysis and our putative AltPLC protein has yet to be achieved. Beyond the obvious development of novel control methods, identification of a phospholipase C protein of independent evolutionary origin is a unique and significant discovery that may ultimately aid in elucidating / refining the evolutionary origins of *Phytophthora*, and give us an insight into the process of independent convergence events in general terms.

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Systemic acquired resistance—a new addition to the IPM clubroot toolbox?

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INTRODUCTION

Clubroot caused by *Plasmodiophora brassicae* affects the Brassicaceae family of plants causing root galling, stunting and wilting of many important vegetable crops. There has been no 'silver bullet' solution to clubroot but a number of 'tools' are available to manage the disease. Integrated use of these 'tools', including detection of *P. brassicae* and prediction of yield loss due to clubroot, identification and elimination of hygiene risks together with in-field cultural methods, use of resistant varieties, manipulation of soil pH, calcium and boron amendment and strategic use of pesticides has been extremely effective in vegetable production systems (1).

Microarray analysis conducted at the early time points during the infection process of *P. brassicae* in *Arabidopsis* (4, 7 and 10 days after inoculation) identified a number of genes and pathways that may regulate disease expression in *Arabidopsis* (2). Manipulation of the salicylic acid (SA) signalling pathway may induce systemic acquired resistance (SAR), a state of heightened defensive capacity in plant species. This paper describes preliminary experiments to study the effect of SA as an inducer of SAR in *Arabidopsis* and broccoli, and assess the potential for SAR to be incorporated into the IPM 'toolbox' for clubroot management.

MATERIALS AND METHODS

A proof of concept study was conducted using *Arabidopsis*. Roots were treated with 0.5 mM SA for 1 minute and then inoculated with *P. brassicae* resting spores 4 hours after treatment. Plants were assessed for disease expression 50 days after inoculation.

A broader range of SA dip rates (1–10 mM) and contact times were evaluated in order to induce SAR in broccoli. Plants were inoculated with a spore suspension of *P. brassicae* 24 hours after treatment and assessed for disease expression 6 weeks after inoculation. A real-time reverse transcriptase quantitative PCR (RT-qPCR) assay was developed to determine the expression of the chitinase gene in broccoli roots and leaves. Biochemical methods are also being developed to confirm SAR induction.

RESULTS AND DISCUSSION

Clubroot disease was strongly suppressed in salicylic acid treated *Arabidopsis* plants (Fig 1). Fifty days post-inoculation SA treated plants had a much lower disease index and infection rate (DI=20, IR=50%) compared to untreated plants (DI=81.5, IR=100%).

A 15 min root dip in 1 mM SA 24 hours before inoculation was the most effective method of SAR induction in broccoli. This treatment consistently increased expression of the chitinase gene by between 2.3 and 5.5 fold in roots and leaves confirming a systemic response. At concentrations in excess of 1 mM SA, changes in the expression of the chitinase gene were less consistent. Frequently these higher concentrations of SA caused a decrease in the expression of the chitinase gene. At the higher rates SA might not be translocated or it may alter the physiology of the plant. A similar result (ie. increased control only at the lowest rate 1 mM) was obtained from disease expression studies using broccoli (Fig 2). SA was phytotoxic to plants at 10 mM.

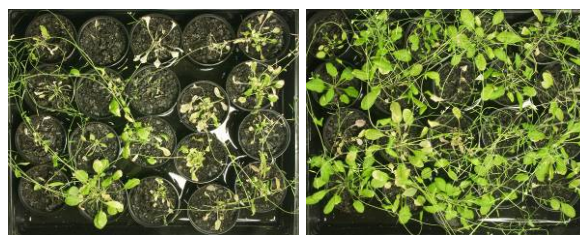


Figure 1. *Arabidopsis* plants 50 days after inoculation with *P. brassicae*. Plants on the right have been pretreated with salicylic acid to induce SAR.



Figure 2. Symptoms of clubroot on roots of broccoli plants 6 weeks after inoculation which occurred 24 hrs after treatment with SA (clockwise from top left 0, 1, 2.5 and 10 mM SA). Each image shows the range of symptoms in each treatment group.

This is the first evidence that SAR induction may be a useful addition to the IPM clubroot 'toolbox'. Work is ongoing to further optimise rates and timing of application of SA, to identify and evaluate other inducers and to extend the work to other pathogens.

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.

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Prevalence and pathogenicity of *Botryosphaeria lutea* isolated from grapevine nursery materials in New Zealand

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INTRODUCTION

Botryosphaeria species are considered important pathogens of grapevines worldwide; they are associated with dieback in mature vines and decline of young vines. They have been isolated from scion and rootstock canes in France and Spain (1, 2). A 2008 survey of nine grapevine nurseries around New Zealand showed that 23% of the canes and grafted plants collected were infected with *Botryosphaeria* spp. From the isolates recovered, 59% were identified as *B. lutea* (unpublished data), making it the most important of the *Botryosphaeria* species to threaten New Zealand vineyards.

This paper reports the distribution and prevalence of *B. lutea* in plant materials from different grapevine nurseries in New Zealand, as well as the variation between nurseries in pathogenicity of their isolates.

MATERIALS AND METHODS

Isolation and Identification of *Botryosphaeria* spp. from nursery plant materials. Plant materials comprising 5–15 samples of each tissue type [apparently healthy grafted plants, failed grafted plants (or Grade 2 plants), scion and rootstock cuttings of different varieties] were collected from 9 grapevine nurseries from different climatic zones in New Zealand. Isolations were made from the surface-sterilised plant samples, with 0.5 cm pieces cut from different parts of each sample placed onto potato dextrose agar with 0.5 g/L streptomycin sulphate (PDAS). Plates were incubated for 72 h and *Botryosphaeria*-like colonies were subcultured onto prune extract agar (PEA) plates to induce sporulation. Isolates were later identified by conidial characteristics and by molecular methods.

Pathogenicity Tests of *B. lutea*. Mycelium plugs from 4 day old *B. lutea* PDA cultures were inoculated onto the wounds created when the shoot tips were cut from rooted one-year-old Sauvignon blanc canes. Four rooted cuttings were used per isolate and control plants were inoculated with sterile agar. The inoculated plants were kept in the greenhouse for 28 days and then the bark peeled off so that the cane lesions could be measured. Re-isolation onto PDAS was done with 1 cm sections of the canes that were cut from 0 to 5 cm beyond each lesion. The plates were incubated in the dark for 72 h at room temperature and assessed for characteristic growth of *B. lutea*.

RESULTS

***Botryosphaeria lutea* Prevalence and Distribution.** *B. lutea* was isolated from seven of the nine nurseries sampled (Table 1). However, one of the two nurseries that were negative for *B. lutea* submitted only part of the sample types requested. The presence of *B. lutea* in different grapevine nurseries was statistically significant using the Pearson Chi-square test ($P < 0.001$).

Botryosphaeria lutea was isolated from all plant materials except Grade 2 plants which comprised only 5% of the total samples collected (Fig. 1). Most isolates (51%) were from 1 cm above and below the graft unions of failed grafted and apparently healthy plants. *B. lutea* infections from the more distant sections of grafted plants occurred in the scion (8%) and rootstock (1%)

materials. Rootstock and scion cuttings had *B. lutea* infections, 18% and 21% respectively, mostly from the middle and basal parts. The distribution of *B. lutea* on different parts of the plant samples was statistically significant using the Pearson Chi-square test ($P < 0.000$).

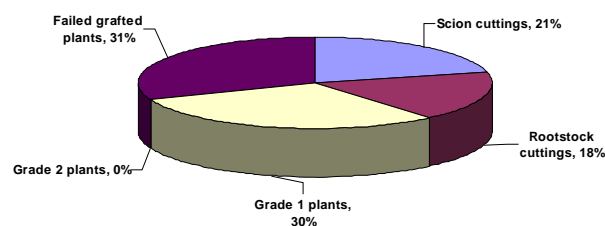


Figure 1. Infection of *B. lutea* on different nursery plant materials.

Pathogenicity. All *B. lutea* isolates were found to be pathogenic to grapevines although their virulence varied between nurseries (Table 1.). Nursery 9 isolates were the most virulent, representing 8% of the total isolates. Nursery 5 had the highest frequency (62%) of *B. lutea* isolations and the isolates from Nursery 7 were the least virulent overall.

Table 1. Incidence of *Botryosphaeria* spp and *B. lutea* on grapevines, and pathogenicity of *B. lutea* isolates based on re-isolation positions along inoculated canes.

Nursery source	<i>B. lutea</i> incidence (% of samples)	% samples with any <i>Botryosphaeria</i> spp. isolated	* Mean infection length (cm)
Nursery 9	16%	31%	10.0 a
Nursery 3	20%	41%	7.3 ab
Nursery 11	3%	26%	5.7 abc
Nursery 10	8%	8%	4.3 bc
Nursery 2	5%	5%	3.9 bc
Nursery 5	60%	63%	3.6 bc
Nursery 7	3%	5%	2.7 c
Nursery 1	0%	5%	
Nursery 4	0%	0%	

* Values within a column with different letters are significantly different at $P < 0.000$

DISCUSSION

This research shows that *B. lutea* is the most prevalent species of *Botryosphaeria* found in nursery plant materials and that their pathogenicity varies between nurseries. *B. lutea* are also commonly found in older grapevines particularly in the North Island (Baskarathevan, J., per. comm.) The presence of *B. lutea* in nursery plant materials indicates that use of infected planting materials or contamination may have occurred in the propagation process and this infestation can carry the disease into new vineyards.

ACKNOWLEDGMENT

This research is funded by Winegrowers New Zealand.

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Infection and disease progression of *Neofusicoccum luteum* in grapevine plants

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INTRODUCTION

Species of the Botryosphaeriaceae are major pathogens of grapevines worldwide that are frequently found to be associated with trunk and cane dieback, internal necrotic stem tissues and bud mortality (3). An accurate monitoring of disease progression is therefore important to evaluate disease susceptibility in grapevine plants. Although some fungi have evolved a variety of morphogenic strategies to enter plants using infection structures such as appressoria (2), the invasion of grapevine tissues by species of the Botryosphaeriaceae mostly occurs through wounds made by pruning or other injuries (unpublished data). Initial pathogenicity experiments indicated that shoots of the major grapevine cultivars grown in New Zealand were equally susceptible to infection, with *Neofusicoccum luteum* being the most prevalent and pathogenic species. The aim of this research was to investigate infection processes and the progression of *N. luteum* infection on grapevine leaves and shoots.

MATERIALS AND METHODS

Conidia of *N. luteum* were obtained by inducing their production on infected, green grapevine shoots (1). The conidial suspensions used for all inoculations were adjusted to a final concentration of 10^4 conidia/mL. The grapevine plants used were 18 months old potted Pinot noir growing in a shade house. Wounding on leaves and shoots was done by scraping the surface layer of a tiny section (2–5 mm²) with a sterile scalpel, after which a drop of the conidial suspension was applied onto both wounded and non wounded sections of attached or detached leaves and shoots. Controls were inoculated with sterile distilled water. There were six replicates for each type of inoculated site.

The detached leaf and stem tissues were observed by SEM 24 h after inoculation. For the attached tissues, plants were grown for 4 months and watered daily. Scanning electron microscopy (SEM) observation or pathogen re-isolation was done on the leaves 24 to 72 h after inoculation and on the shoots at monthly intervals for 4 months. Pathogen re-isolation from the shoots tissues were taken at 1 cm intervals below and above the inoculation point.

RESULTS

No infection occurred in any non wounded shoots or leaves (attached or detached); no fungal cultures characteristic of *N. luteum* were isolated from these tissues. The SEM observations of inoculation sites on the unwounded attached leaves revealed no conidia on the surfaces at 24 h after inoculation. However, on the surfaces of the attached (wounded) shoots and leaves, the conidia were observed to have germinated, with the germ tubes penetrating into the wounded tissue at 24 h after inoculations (Fig. 1A). On the detached (wounded) shoots and leaves, there were networks of mycelium at 24 h after the inoculations. Re-isolation from these tissues yielded 100% *N. luteum*.

Further SEM of the pathogen at 3 months after inoculation onto the wounded shoots showed long threads of mycelium growing in through the vessels (Fig 1B). Detection of pathogen movement by monthly re-isolation at 1 cm intervals below and above the inoculation points showed pathogen progression increased with time, being greater in the upward than the downward direction (Fig. 2).

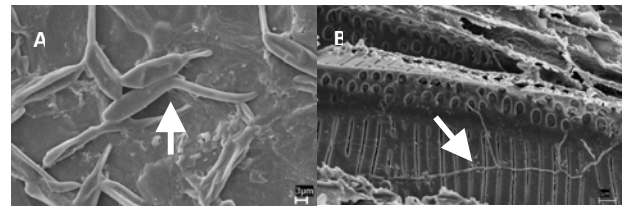


Figure 1. Development of *N. luteum* on wounded grapevine tissue (A) arrow shows conidia germinating on an attached leaf surface after 24 h, (B) mycelium growing through shoot xylem vessel 3 months after inoculations.

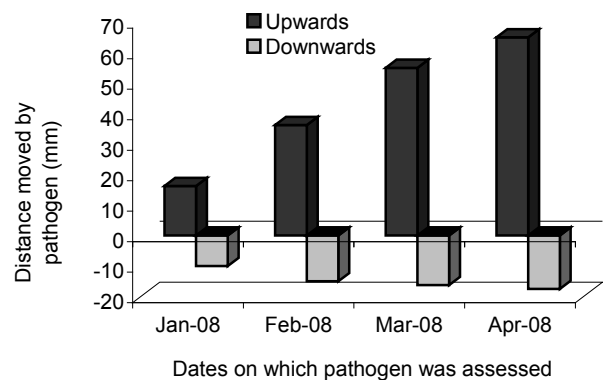


Figure 2. Recovery of *N. luteum* from tissue above and below wounded inoculation sites of 18 months old Pinot noir grapevines.

DISCUSSION

No infection by *N. luteum* conidia was seen on non wounded tissue and no conidia were observed at 24 h after the inoculations on non wounded (attached) leaf surfaces. This suggests that the conidia could not attach to the surfaces and were lost from them. The faster development of germinating conidia on the attached shoots and leaf surfaces (wounded) than on the detached tissues at 24 hr after infection could be due to active inhibitory plant-pathogen interactions. This may also explain why plants under water stress had enhanced susceptibility to infection. In stems of 18 months old vines, movement of the pathogen was faster in the upward than downward direction, possibly because it followed xylem flow, SEM observations showed pathogen presence in xylem vessels, which may allow for the non-symptomatic progression previously observed (unpublished data)

ACKNOWLEDGEMENTS

The authors gratefully acknowledge funding from the New Zealand Winegrowers Inc.

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Carbohydrate stress increases susceptibility of grapevines to *Cylindrocarpon* black foot disease

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INTRODUCTION

Stress can predispose woody plants to pathogen infection, increasing both disease incidence and severity (1). Defoliation by means of leaf plucking can stress a plant by decreasing the availability and concentration of photosynthate, compromising its resistance to other biotic and abiotic stresses (2). This experiment investigated the effect of carbohydrate stress, as induced by leaf plucking of grapevines, on the disease severity and incidence of black foot disease, a serious threat to vineyards around the world. This disease is caused by *Cylindrocarpon* species including *C. destructans*, *C. macrodidymum*, and *C. liriiodendri* (3).

MATERIALS AND METHODS

Plants (1 year old) of Sauvignon Blanc scion wood grafted to rootstocks 101–14 or Schwarzmann (ten per treatment), were grown in a 50/50 mix of vineyard soil and potting mix. Pots were laid out in a completely randomised design in a greenhouse in September 2006. Leaves were plucked (Nov 2006) from scion shoots above the fourth node, to induce three different levels of carbohydrate stress, being none (level 0; no removal), moderate (level 1; every third leaf removed) and high (level 2; every third leaf was left). Plucking treatments were performed three times at three weeks apart on new shoot growth and then the root systems of all the vines were wounded by slicing down into the soil, four times around each plant. For each stress level, half the plants were inoculated with 50 mL (per plant) of a mixed (three *C. destructans* isolates) conidial suspension (10^6 /mL) poured over the soil surface followed by 50 mL of tap water. The remaining plants were treated with 100 mL of tap water.

Plants were grown for a further six months prior to assessment. Root and shoot dry weights were recorded and isolations made by plating sections of surface sterilised trunk tissue onto potato dextrose agar. Plates were incubated at 20°C for 7 d and assessed for the presence of *C. destructans* colonies (3).

RESULTS

Cylindrocarpon destructans disease severity and incidence was similar for both Schwarzmann (8.4% and 29.3%, respectively) and 101–14 rootstocks (14.9% and 31.0%, respectively). Although not significant, the highest disease severity was seen with high carbohydrate stress compared with moderate or no stress (Table 1). However, when data for the moderate and no stress treatments were combined (because the effects were similar), the disease severity was significantly higher for the highly stressed plants ($P=0.043$). Stress did not influence disease incidence ($P=0.551$). Infection also occurred in the un-inoculated plants, due to the soil being infested by *Cylindrocarpon* spp., but disease severity was higher in the plants inoculated with *C. destructans* (40.5%) than those that were not (19.5%).

Root dry weights were similar in inoculated and uninoculated plants, but were significantly lower for highly stressed plants (15.6 g) than both the moderately stressed (19.9 g; $P=0.000$) and unstressed plants (18.5 g; $P=0.003$). An interaction between inoculation and stress ($P=0.031$) showed that inoculated and highly stressed plants had the lowest root dry weight (14.9 g). Root dry weights differed ($P=0.0001$) between the rootstock

varieties, 101–14 (19.2 g) and Schwarzmann (16.8 g), which responded differently to stress ($P=0.062$), being 17.9 g and 13.2 g, respectively in the highly stressed treatment. There was no significant effect of carbohydrate stress ($P=0.259$) or inoculation ($P=0.885$) on shoot dry weight

Table 1. Effects of the three carbohydrate stress treatments (analysis a), and two stress treatments (0+1 compared with 2; analysis b) on *C. destructans* disease severity (% infected wood pieces) of grapevines. Data are combined averages for inoculated and uninoculated plants for two rootstock varieties.

Stress level *	Disease severity	Stress level **	Disease severity
0:none	7.15	0 + 1	7.60
1:moderate	8.15		
2:high	19.65	2	19.65
<i>P</i> value	<i>P</i> =0.138		<i>P</i> =0.043

*Analysis a

**Analysis b

DISCUSSION

This study showed that carbohydrate stress caused by leaf plucking significantly increased the severity of black foot disease and decreased root dry weight. There was also some indication, although not significant, that Schwarzmann was more affected by carbohydrate stress than 101–14. These results are relevant to the industry since canopy thinning is a regular practice in the vineyard. Hunter *et al.* (4) reported that no negative effects were thought to be associated with the practice; however, in light of these results care should be taken with deciding the intensity of canopy thinning in areas at risk to *Cylindrocarpon* infection.

ACKNOWLEDGEMENTS

New Zealand Winegrowers and Lincoln University for funding this project.

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Botryosphaeria spp. associated with bunch rot of grapevines in south-eastern Australia

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INTRODUCTION

Species of *Botryosphaeria* are common wood pathogens of grapevines and are responsible for the disease known as 'Bot canker' (1). Recently some species have also been implicated in bunch rot of *Vitis vinifera* in Australia (2). While pathogenicity tests have been conducted on grapevine wood for several species, it is unknown which of these infect bunches and how they enter the berry.

MATERIALS AND METHODS

Plant survey. Between 2007 and 2009 two vineyards in the lower Hunter Valley, NSW, were sampled for species of *Botryosphaeria*. Samples were collected from 200 each of Chardonnay and Shiraz grapevines symptomatic of Bot canker at different phenological stages: dormant buds (B), flowers (F), pea-sized berries (P) and berries at harvest (H). Samples were also collected from the margin of healthy and discoloured internal wood (W) from the trunks of each plant.

Fungal isolation and identification. Samples were surface sterilised, placed onto Potato Dextrose Agar (PDA) amended with Streptomycin Sulfate and incubated at 25°C in the dark. Fungal cultures characteristic of *Botryosphaeria* spp. were sub-cultured onto PDA and/or triple autoclaved pine needles on 1% water agar maintained under near UV light (12hr light/dark) to encourage sporulation. *Botryosphaeria* spp. were identified according to spore morphology and sequencing of the rDNA internal transcribed spacer region.

Pathogenicity tests on berries. Disease-free, surface sterilised Shiraz and Chardonnay berries at harvest were inoculated with 10 µL spore suspensions from 19 isolates belonging to the species listed in table 1. Conidial suspensions at concentrations of 10⁶ and 10⁴ spores/mL were used in trial 1 and 2, respectively. Control berries were inoculated with 10 µL of sterile distilled water. The berries were incubated in 24 well plates at 27°C in the dark for 15 days. A constant relative humidity was maintained by the addition of 20 mL of sterile water to each plate. Disease incidence (%) and severity (1–10) was recorded for each treatment. A disease index (DI) was calculated for each treatment replicate at each time of recording:

$$DI = \frac{\sum \text{diseased berries} \div \sum \text{total berries} \times \sum \text{disease severity scores}}{\sum \text{max disease severity scores}}$$

Pathogenicity tests on canes. Detached one year old canes were inoculated with 4 mm diameter mycelium plugs of 14 *Botryosphaeria* isolates, previously tested on berries, by inserting the plugs into the wood. Canes were incubated on moist filter paper in Petri dishes at 27°C in the dark. After 15 days, lesion lengths were measured for each isolate.

RESULTS

Survey and fungal identification. To date, a collection of 177 isolates of *Botryosphaeria* spp. has been established. The species isolated included *Diplodia seriata*, *Botryosphaeria dothidea*, *Neofusicoccum parvum*, *N. luteum*, *Dothiorella viticola*, *Diplodia*

mutila and *Lasiodiplodia theobromae*. Abundance and origin of each species are listed in Table 1.

Table 1. *Botryosphaeria* spp. isolated from grapevine tissue.

Species	Origin tissue				
	B	F	P	H	W
<i>D. seriata</i>	83	2	3	6	27
<i>B. dothidea</i>	15	0	0	3	7
<i>N. parvum</i>	7	1	0	2	4
<i>N. luteum</i>	3	0	0	2	2
<i>D. viticola</i>	3	0	0	0	2
<i>D. mutila</i>	0	0	0	0	4
<i>L. theobromae</i>	0	0	0	0	1

Pathogenicity tests All isolates produced bunch rot symptoms on berries including the formation of mycelia and pycnidia, darkening of the berry skin, oozing and berry collapse. Disease indices for each replicate treatment varied significantly from control berries. Variation in the rate of increase of the disease index was detected within and between species. Inoculation of canes showed lesion development and pycnidia formation on the cane surface. There were significant variations in lesion lengths between species. In both berry and cane pathogenicity tests, virulence appeared to be independent from the origin of the isolate.

DISCUSSION

Results suggest that *Botryosphaeria* spp. have the potential to contribute to grapevine bunch rots and infect grapevine canes. *Botryosphaeria* spp. appear to be non-tissue specific in their pathogenicity toward grapevine. Trials are in progress to establish if these results can be reproduced under field conditions and whether the infection of buds or flowers by *Botryosphaeria* results in bunch rot at harvest.

ACKNOWLEDGEMENTS

The authors would like to thank Chris Haywood for assistance with field sampling and the growers for access to their vineyards. This work was supported by a National Wine and Grape Industry Centre (NWGIC) Scholarship and through the Wine Growing Futures program, a joint initiative of the Grape and Wine Research and Development Corporation and the NWGIC.

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Honey bees—do they aid the dispersal of *Alternaria radicina* in carrot seed crops?

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INTRODUCTION

A. radicina is a seed and soil-borne pathogen of carrot (*Daucus carota*) crops now common in Canterbury, New Zealand. *A. radicina* causes black rot disease, but can also reduce seed germination and seedling establishment. For carrot seed production, honey bees (*Apis mellifera*) are introduced into the crop to improve pollination. During this process, if bees visit *A. radicina* infected umbels and pathogen spores adhere to them, they may disperse the pathogen within the crop.

MATERIALS AND METHODS

To test this hypothesis, both live and dead bees were collected from around beehives that had been placed in three seed crops in the Ashburton region, Canterbury, NZ. Fifty bees were selected at random, placed in 100 ml sterile distilled water in a 250 ml bottle and shaken using a Wrist action shaker (Griffin) at maximum speed (1000 rpm) for 15 min to dislodge any spores on the cadavers. The suspension was filtered using sterile Whatman paper No. 105 and the filtrate centrifuged in 50 ml tube at 4000 rpm for 5 min. The supernatant was discarded, the pellet reconstituted in 100 µl water and plated on *A. radicina* semi selective agar and incubated at 27°C. After 7 d fungi growing on these plates were isolated into pure culture. The isolated fungi were identified by morphological, cultural and sporulation characteristics. To confirm those cultures preliminarily identified as *A. radicina*, the fungal DNA was obtained using Puregene® DNA purification kit protocol. The universal primers ITS 4 and ITS 5 were used to amplify a portion of the rDNA. For each PCR reaction 1 µl of DNA (10 ng/µl) was mixed with 24 µl of PCR mixture containing the manufacturer's buffer, 200 µM of each dNTP, 1.5 mM MgCl₂, 5 pmol of each primer, 1.25 U Faststart Taq (Roche). A negative control without DNA template was also included. Amplification was done using a Mastercycler® Gradient (Eppendorf, USA) using the following thermal cycles: initial denaturation at 94°C for 3 min then 35 cycles of: denaturation at 94°C for 2 min, annealing at 57°C for 30 s and extension at 72°C for 1 min for 35 cycles following final extension at 72°C for 10 min and hold at 4°C. The PCR product was separated by electrophoresis on a 1% agarose gel in 1X TAE buffer and visualised using ethidium bromide dye under UV light (Versadoc Imaging Systems Model-3000; Bio-Rad, USA). The PCR product was sequenced in 3130 xl genetic analyser (ABI Prism, Applied Biosystems) and the obtained sequence of the rDNA was then compared with sequences present on GenBank (<http://www.ncbi.nlm.nih.gov/>) using a Blast search to confirm its identity.

RESULTS

In addition to *A. radicina*, a few other fungal colonies were also grown on selective agar medium. Honey bees from different carrot fields carried different amounts of inoculum of *A. radicina* on their bodies. The average numbers of colonies recovered from 50 bees were ~166 and spore per bee ratio was 3.3:1. The electrophoresis resulted in a single band of ~600 bp on the agarose gel (Fig 1). The DNA sequence obtained from this band was confirmed using Blast as *A. radicina*. The sequence had maximum similarity (100%) with accession numbers AY154704.1, DQ394073.1 and EU807870.1. The sequence from the honey bee

(597 bp) was deposited in GenBank as accession number FJ958190.

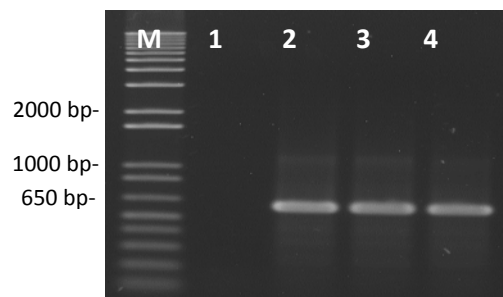


Figure 1. Agarose gel electrophoresis of the amplified rDNA region of *A. radicina* colonies recovered from honey bee bodies collected from three infected fields. Lane M contains 1 kb plus DNA ladder (Invitrogen); lane 1, negative control; lane 2, infected site A; lane 3 infected site B; lane 4, infected site C.

DISCUSSION

The detection of *A. radicina* conidia (38 x 19 µm in size) on the body of honey bees suggests that they have the potential to play a role in the spread of the disease within and among the carrot seed crops. This research supports that of many past researchers that concluded some other insects and mollusc, like flea beetles (1) and slugs (2) in cabbage and, pollen beetles and seed pod weevil (3) in oil rape were able to play an important role in dispersal of *Alternaria* spp. Future work is now focused on quantification of *A. radicina* through real time PCR and the identification of other fungal species carried on the body of honey bees.

ACKNOWLEDGEMENTS

The Foundation for Research Science and Technology and Midlands Seed Ltd funded this research.

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Translating research into the field: meta-analysis of field pea blackspot severity and yield loss to extend model application for disease management in Western Australia

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INTRODUCTION

Blackspot or ascochyta blight, caused predominantly by *Mycosphaerella pinodes*, is the most destructive foliar pathogen of field peas and causes considerable yield loss. The amount of yield loss is mainly driven by primary infection, i.e. spread of wind-borne ascospores from infected pea stubble of previous seasons' crops. In this paper, we present a meta-analysis to show observed disease severity and associated yield loss in Western Australia (WA), explore the feasibility of chemical control, and describe the development and application of a weather-based model to manage the disease.

MATERIALS AND METHODS

The meta-analysis, using different forms of regressions, was done using data from 14 experiments conducted at 13 sites over 8 seasons in WA. The model, "Blackspot Manager" was developed using daily weather data to predict the timing of onset, and progression, of ascospore maturity (Salam et al., 2006). The model was tested with independent field data from agricultural regions in WA. A system was developed to provide model output to the agribusiness community and farmers via internet (<http://www.agric.wa.gov.au/cropdiseases>).

RESULTS

Potential blackspot severity decreases as the season progresses and can be quantified as a function of time of sowing (results not shown). Under the "No control" scenario, the potential disease severity remains above rating 2 until early July (Fig. 1); however, it varies between regions (data not shown). Application of an in-furrow fungicide cannot decrease the severity below rating 2 before mid-June; and fortnightly sprays not before mid-May.

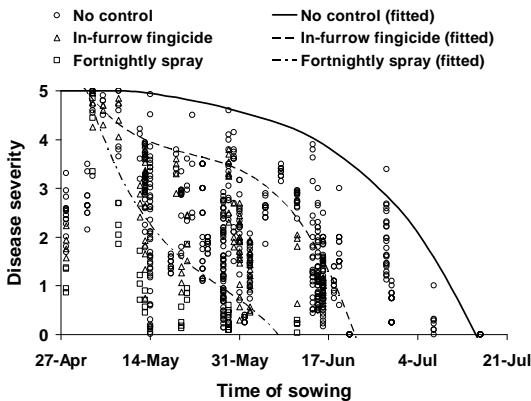


Figure 1. Fitted curves of potential severity of blackspot disease on field pea across WA in response to no control, in-furrow fungicide or fortnightly fungicide spray.

Potential yield reduction does not occur before disease severity rating 1 (Fig. 2). At disease severity rating 2, it reaches around 15%, whereas, the maximum rating of 5 corresponded to a potential yield reduction of around 55%.

The Blackspot Manager uses the pre-season temperature and rainfall to forecast the onset and progression of ascospore release from infected field pea stubble prior to and during the

growing season. Disease severity rating does not exceed 2 until crop has been exposed to more than predicted 40% of seasonal ascospores (Fig. 3).

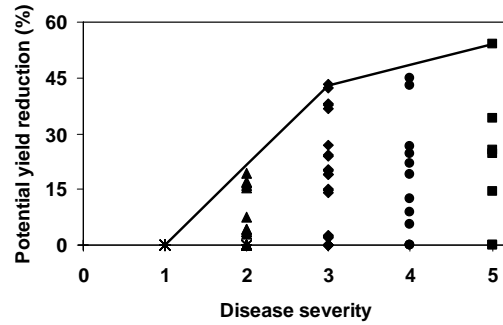


Figure 2. Relationship between blackspot disease severity ratings and potential yield reduction in field pea.

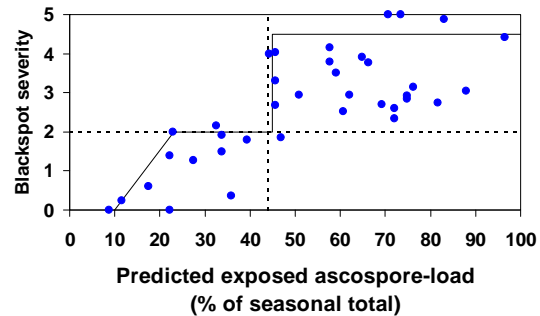


Figure 3. Relationship between predicted exposures to ascospores from infected stubble and disease severity rating at flowering on standing field pea crops.

CONCLUSION

This research shows that chemical control for blackspot management is unlikely to be economical. Consequently, growers must rely on appropriate sowing dates. The outcome of this work is a model that predicts the temporal ascospore-load and allows for the determination of a sowing date that minimises potential yield loss.

ACKNOWLEDGEMENTS

We thank the Australian Grains Research and Development Corporation (GRDC) the Department of Agriculture and Food Western Australia for supporting this research.

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Development of a model to predict spread of exotic wind and rain borne fungal pests

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INTRODUCTION

Exotic fungal plant pathogens pose a threat to Australian agriculture. Some of the most devastating fungal pathogens are transported by rain splash, wind dispersal or a combination of both. The transport of fungal pathogens via rain and wind makes containment and eradication difficult. *Ascochyta rabiei*, causal agent of ascochyta blight of chickpea, is a wind/rain borne pathogen already present in Australia. *A. rabiei*, therefore, provides a suitable pathogen for modelling the potential spread of an exotic fungal pathogen dispersed by wind and rain.

MATERIALS AND METHODS

Field trials and laboratory studies were conducted to examine key environmental factors influencing the short distance (rain splashed) and long distance (wind borne) distribution of spores.

Field trials. were undertaken at Kingsford Research Station, 53 km north-east of Adelaide in 2007 (S 34.54521, E 138.78117), and at Turretfield Research Station, approximately 60 km north-east of Adelaide in 2008 (S 34.54760, E 138.82225). Plots (11 x 11 m) at each site were planted with 3 cultivars of chickpea; Howzat (moderately susceptible), Almaz (moderately resistant) and Genesis 090 (resistant) (Figure 1). Infested stubble was placed at the centre of each plot and disease spread was recorded weekly. The percentage of plants infected in 1 m² quadrats and the number of growing points (number of main stems and branches) for three plants of each cultivar were recorded over time. Weather data were collected via an automated weather observation system at the Roseworthy, within 13 km of the field sites.

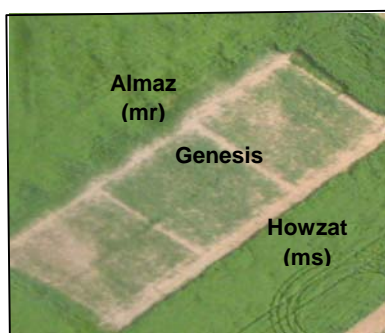


Figure 1. Aerial photograph of 11 x 11-m chickpea plots at Turretfield in 2008. Top, Almaz with moderate disease incidence; middle, Genesis 090 with no disease; bottom, Howzat with severe disease.

Laboratory experiments. were conducted to investigate the effect of wind speed (m/s), rain splash (ml/m) and a combination of the two factors on the dispersal of conidia in a purpose-built wind and rain tunnel.

Model development. A model for determining the spread of rain and wind-borne pathogens was developed for ascochyta blight based on the spatiotemporal model for simulating the spread of anthracnose in lupin fields (1). The data collected from the field trials and laboratory experiments were entered as the model

parameters. The adjusted parameters produced a model output in Mathematica™ that best fit field disease observations.

RESULTS

The model. For (a) number of growing points (Figure 2) collected from the 2007 field experiments and (b) spore dispersal in wind and rain tunnel experiments were used as parameter inputs for the model. Plant infection data from the 2007 field experiments were compared and calibrated with simulations run in the model. Data for disease incidence and severity in the 2008 field trial were then used to validate the model.

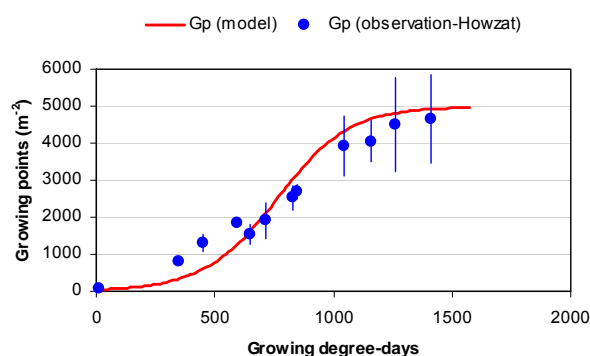


Figure 2. Growing points (Gp, main stems and branches) influenced by degree days as predicted by the model and compared to field observations in chickpea cv. Howzat at Kingsford 2007.

DISCUSSION

The outcome of this work is a model calibrated with experimental data and tested with field observations for accuracy. Weather data are entered into the model and pathogen spread is predicted by graphical output showing disease occurrence in the field. The number of conidia produced per lesion on each of the cultivars will be estimated, to add more information to the model. When fully developed, the model will provide a basis for predictive models for exotic plant pathogens. It will also facilitate improved management of disease through forecasting, and more precise application of fungicide and timing of crop sowing.

ACKNOWLEDGEMENTS

We thank the CRC for National Plant Biosecurity, the University of Adelaide, South Australian Research and Development Institute, and Department of Agriculture and Food Western Australia for supporting this research.

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Psyllid transmission of huanglongbing from naturally infected Shogun mandarin to orange jasmine

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INTRODUCTION

Huanglongbing (HLB), previously known as citrus greening is a devastating disease of citrus. It affects all citrus cultivars and causes rapid decline of trees. HLB is caused by a phloem limited fastidious bacterium, *Candidatus Liberibacter* a gram negative bacterium belonging to alpha proteobacteria (1). At least 3 species of *Candidatus Liberibacter* have been reported to be associated with HLB, *Ca. L. africanus*, *Ca. L. asiaticus*, and *Ca. L. americanus*. The two *Ca. Liberibacter* species, *africanus* and *asiaticus*, are transmitted by the psyllid vectors *Trioza erytreae* (Del Guercio) in Africa and *Diaphorina citri* (Kuwayama) in Asia, respectively.

Candidatus Liberibacter can infect nearly all citrus species, cultivars and hybrids. Later, orange jasmine, *Murraya paniculata* (L.) Jack and Chinese box orange, *Severnia buxifolia* (Poiret) Men., have been reported to harbor HLB pathogen (2, 3).

This paper reported transmission of *Ca. L. asiaticus*, Asian form HLB, by its psyllid vector, *D. citri* from naturally infected Shogun mandarin, *Citrus reticulata* Blanco, to orange jasmine (*M. paniculata*).

MATERIALS AND METHODS

Insect vector. Adults of disease free *D. citri* were caged on naturally infected Shogun mandarins grown at Prince of Songkla university experimental plot for one month. The vectors were then used in transmission test.

Transmission test. The insect vectors were released to feed on healthy *M. paniculata* seedlings (test plants) using 0, 1 and 15 insects per test plant. Healthy test plants were also placed under infected Shogun mandarin trees to obtain natural transmission.

Detection of HLB pathogen. Total DNA of *M. paniculata* plant was extracted from 0.1–0.5 g of leaf midribs using CTAB method (3). PCR detection of *Ca. Liberibacter asiaticus*, HLB pathogen, was carried out with primers specific to 16S rRNA gene of Asian HLB (1).

RESULTS AND DISCUSSION

D. citri successfully transmitted HLB pathogen from the naturally infected shogun mandarin to *M. paniculata* even by single insect vector (Table 1). Transmission occurred within 7 and 9 weeks after released the insect vector on *M. paniculata* test plant using 1 and 15 insects, respectively. For natural transmission (NT, placing test plants under diseased mandarin tree), test plant became infected within 28 weeks. Single insect transmission rate was as high as by 15 insects (50%) but it was slightly lower than the natural transmission (75%). All of HLB infected *M. paniculata* by insect vector showed typical symptom (Fig 1) resembling HLB infected citrus (3). HLB pathogen as tested by PCR remained in infected *M. paniculata* with typical symptom more than 30 weeks which was contrast to a previous report (4).

Table 1. Transmission of HLB pathogen (*Ca. L. asiaticus*) by *D. citri* insect vector.

No. of insect ¹	No. of transmission /total test plant ²	Transmission rate (%)
0	0/4	0
1	2/4	50
15	2/4	50
NT	3/4	75

¹ Number of *D. citri* insect vector feeding on one test plant (healthy *Murraya paniculata*), NT (natural transmission), healthy test plant placed under infected shogun mandarin.

² Transmission determined by HLB symptom on test plant and followed by PCR amplification of HLB DNA.



Figure 1. Infected *Murraya paniculata* showing typical HLB symptoms (interveinal chlorosis and blochy mottle leaves)

A by single *Diaphorina citri* insect vector

B by 15 insect vectors

C by natural transmission

ACKNOWLEDGEMENTS

We wish to thank Thailand Research Fund (TRF) and Nakajima Peace Foundation for financial support and also to Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University, for providing laboratory facilities.

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Transmission of ‘*Candidatus Phytoplasma australiense*’ to *Cordyline* and *Coprosma*

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INTRODUCTION

Phytoplasmas are specialised plant pathogenic bacteria (class Mollicutes) that live in the phloem tissue of host plants and in the tissues of phloem-feeding insects that transmit them. The phytoplasma “*Candidatus Phytoplasma australiense*” is associated in New Zealand with diseases of three naturally occurring host species, New Zealand flax, (*Phormium tenax*, family Phormiaceae), New Zealand cabbage tree (*Cordyline australis*, family Laxmanniaceae), and karamū (*Coprosma robusta*, family Rubiaceae), as well as one cultivated host, strawberry (*Fragaria x ananassa*, family Rosaceae). Previous work (4, 5) has established that “*Ca. P. australiense*” can be transmitted from *Phormium* to *Phormium* by the New Zealand flax planthopper *Zeoliarus (Oliarus) atkinsoni* (family Cixiidae). In this study, we have identified that a second species in this genus, *Zeoliarus oppositus*, acts as a vector between two of the naturally occurring hosts.

MATERIALS AND METHODS

Small saplings of *Cordyline australis* and *Coprosma robusta* were grown from seed. *Z. oppositus* adults were collected from the wild by ‘beating’ symptomatic and non-symptomatic plants of *C. robusta*. Transmission experiments were set up by caging 10 individuals of *Z. oppositus* onto seedlings of the test species (10 replicates).

DNA was extracted from various plant tissues and whole insects. Phytoplasma presence in insects and plant samples was examined by one stage and nested PCR using the “universal” phytoplasma 16S primers (P1+P7, R16F2 + R16R2) (1).

RESULTS

Insect donors. *Zeoliarus oppositus* adults collected from the donor population in early summer were examined for the presence of phytoplasma using one stage PCR. A total of 4 were positive from 33 individuals examined, indicating an infection rate of c. 12%. Other adults were caged onto *Cordyline* and *Coprosma* seedlings. Over 60% were still alive on removal at 3–4 weeks.

Cabbage tree. Four of the ten plants exposed to *Z. oppositus* developed symptoms within one year. Symptoms closely resembled those of ‘initially-affected’ tufts of diseased trees observed in the field (1). At least one of the tissue samples from all 4 symptomatic plants were positive using one stage PCR (Table 1).

Karamū. After one year, 8 of the 10 *Coprosma* plants exposed to *Z. oppositus* showed leaf reddening of older leaves and one of these also showed dieback of the main shoot, symptoms consistent with phytoplasma infection in this host (3). Two plants that had been exposed to *Z. opposites* tested positive for phytoplasma.

Table 1. Phytoplasma detection in tissue samples from symptomatic cabbage trees.

Plant	Days after insects caged	Shoot apex	Leaf bases	Rhizome apex
Ca7	89	+	not tested	+
Ca2	131	+	+	+
Ca1	164	-	+	-
Ca9	196	+	+	-

DISCUSSION

We conclude that *Z. oppositus* is a vector of “*Ca. P. australiense*”, capable of transmitting this phytoplasma from *Coprosma* to both *Cordyline* and *Coprosma*.

Both species of *Zeoliarus* are endemic to New Zealand. The ecology of *Z. oppositus* is consistent with the proposal that it is a vector for “*Ca. P. australiense*”. It is a very common species where it is found in natural and modified habitats throughout the country. Given the polyphagous nature of *Z. oppositus*, it is apparent that it may transmit the phytoplasma to other plants.

ACKNOWLEDGEMENTS

This work was funded by New Zealand’s Foundation for Research, Science and Technology and Ministry of Research, Science and Technology. We thank Lia Liefting and John Charles for useful discussions.

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Australian grapevine yellows phytoplasma found in symptomless shoot tips after a heat wave in South Australia

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INTRODUCTION

In 2008/09, extremely high incidences of Australian Grapevine Yellows (AGY) in *cv.* Riesling vineyards at Nildottie, near Loxton, South Australia, suggested incursion of a new yellows pathogen. To investigate, in March 2009 we surveyed vineyards, tested material via PCR and examined the role of a period of very high temperature on symptom expression. The findings challenged our previous theory on the pathogenesis of AGY.

MATERIALS AND METHODS

Vineyard Surveys. In Nildottie vineyards (Table 1), 50 vines/block, ≥ 3 blocks/transect and ≥ 3 transects /vineyard were scored for typical AGY *viz.* yellowed, downward curled leaves, unglified shoots and shrivelled bunches.

PCR-Tests. Following a heat wave in Jan-Feb 2009 with 13 consecutive days $\geq 38^\circ\text{C}$ ($5 \geq 42^\circ\text{C}$), ~ 5 shoots with typical AGY symptoms were selected from each of several cultivars (Table 2) for replicated two-step PCR analysis with both positive and negative controls to detect AGY phytoplasma (AGYp) as per (1,2). Samples were taken from: 1) mature, yellowed leaves; and 2) symptomless new growth on shoot-tips.

RESULTS AND DISCUSSION

Vineyard Surveys. Previous levels of AGY in the Riesling vineyards at Nildottie had averaged 3–5% vines but, in 2008/09, the incidence was extreme and unprecedented in Australia (Table 1). Adjacent, usually symptomless red cultivars were also diseased.

Table 1. Incidence of AGY in vineyards of several cultivars, Nildottie, South Australia, March 2009.

Scores of AGY Incidence					
Vineyard	# Vines	% AGY	Vineyard	# Vines	% AGY
Riesling 1	562	99.8%	Chardonnay 1	292	1.7%
Riesling 2	366	94.8%	Chardonnay 2	146	Nil
Riesling 3	388	88.9%	Shiraz ¹	150	3.3%
Sangiovese ¹	150	19.3%	Sauv. Blanc	150	Nil

¹ From conservative assessment—actual incidence was likely higher.

The highest incidence we had previously seen on any cultivar was 86% (on Riesling in Renmark, SA, in 1978/79). At Nildottie, the severity of disease was also extreme. Whereas usually only 3–5 shoots/vine are affected by AGY, in 2008/09, most shoots were diseased and in Riesling Vineyards 1 and 2, crop loss was complete.

PCR-Tests. AGYp were found in a high proportion of samples (Table 2). This was consistent with previous tests (data not shown) and counteracted the idea that incursion of a new pathogen caused the extreme disease.

Why were levels of AGY so high in 2008/09? Two possibilities are: 1) Severity: Seasonal increases in temperatures from autumn to early spring in 2008 may have increased the multiplication of overwintering AGYp, raising their titre and so the severity of symptoms seen in 2008/09. In March 2008 at Loxton, mean max./min. temperature (T) (at $32.7^\circ\text{C}/12.5^\circ\text{C}$

respectively) were warmer compared with March averages ($28.2^\circ\text{C}/11.8^\circ\text{C}$).

Table 2. PCR-tests of AGY-affected shoot material and new tip-growth in several cultivars after a heat wave, Nildottie, South Australia, March 2009.

Vineyard ¹	PCR-Tests for AGYp			
	# Shoots with AGYp	# Shoots Tested	# Shoot Tips with AGYp	# Shoot Tips Tested
Riesling 1	4	5	4	4
Riesling 2	5	7	4	6
Riesling 3	4	6	1	5
Sangiovese	1	1	-	-
Shiraz ²	1	1	-	-
Sauv. Blanc	0	1	-	-

¹ The same vineyards as in Table 1. ² Weakly positive for AGYp.

Elsewhere in 2008/09, surveys also showed higher severity but little new incidence (data not shown). 2) Incidence: The higher incidence at Nildottie suggests an increased activity of AGYp vector(s) but raises the possibility of an incursion of a more infective vector(s).

Why the observed remission of symptoms? New growth of AGY affected shoots 10–14 days after heat waves has been seen many times (data not shown). Symptomless new shoot growth produced after the 13-day heat wave of Jan-Feb 2009 was PCR +ve for AGYp (Table 2), suggesting that not all AGYp were killed or denatured by the heat. Hot water treatments (e.g. 45 minutes at 50°C) delivering $\sim 40^\circ\text{C}\text{-hrs}$ /treatment at 50°C or $\sim 200^\circ\text{C}\text{-hrs}$ at 45°C , are used in Europe to reduce transmission rates of FD and other yellows pathogens (3). The 2009 heat wave delivered a lesser though similar heat treatment to the vines at Nildottie. A 46°C max T delivered $\sim 180^\circ\text{C}\text{-hrs}$ at $\geq 45^\circ\text{C}$ and a 45°C max T delivered $\sim 230^\circ\text{C}\text{-hrs}$ at $\geq 43^\circ\text{C}$. We had supposed that these temperatures were lethal to AGYp and thus triggered the observed new shoot growth but this theory is now questioned.

AGY-affected shoots show signs of disturbed phloem cell function, hormone imbalance and deposition of callose in sieve cells (data not shown) but these were insufficient to prevent significant reactivation of shoot growth soon after the heat wave. If AGY symptoms were the result of that damage, the swift 'remission' of AGYp-infected and severely diseased shoots raises conjecture as to the cause of AGY symptoms.

ACKNOWLEDGEMENTS

Dr David Cartwright, Primary Industries and Resources SA, provided some financial assistance for the PCR-tests.

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Association of phytoplasmas with papaya crown yellows disease—a new disease of papaya in Northern Mindanao, Philippines

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INTRODUCTION

“Papaya crown yellows” (PCY) disease of unknown etiology was first observed in Northern Mindanao, Philippines in 2001. PCY was thought to be associated with phytoplasma because its symptoms are similar to phytoplasma diseases of papaya in Australia. The occurrence of PCY poses a potential threat to the papaya industry in Northern Mindanao as well as the whole country. Thus, this research aimed to investigate the etiology of the PCY disease and to determine its identity using sensitive molecular and genetic techniques.

MATERIALS AND METHODS

Sample Collection. Seventy symptomatic and 33 symptomless papaya leaf samples were collected at 8 different locations in Northern Mindanao in April-June 2004. An additional 12 symptomless samples were collected at Los Baños, Laguna province in July 2004 where PCY disease was not observed.

Detection of Phytoplasmas from Papaya DNA using Polymerase Chain Reaction (PCR). DNA was isolated from papaya leaf samples using phytoplasma enrichment nucleic acid extraction method by Dellaporta (1). Universal primer pair fu5/rU3 (2) of the 16S rRNA gene was used for general detection of phytoplasmas in papaya DNA. The group-specific primer pair Tuf f40 and Tuf r1150 (Streten, unpublished) that amplifies 1110 bp of the *tuf* gene was used to detect papaya dieback phytoplasma *Candidatus* *P. australiense* in papaya DNA. *Ca. P. aurantifolia* (TBB strain) and *Ca. P. australiense* were used as positive controls while healthy papaya DNA and sterile distilled water (SDW) were used as negative controls.

Restriction Fragment Length Polymorphism (RFLP) Analysis. PCR products were digested using 1U of *TaqI* restriction enzyme (Biolabs, Australia) and were incubated overnight at 65°C. DNA fragments were separated by electrophoresis in an 8% polyacrylamide gel and visualised by staining with 5% ethidium bromide. *Ca. P. aurantifolia* (TBB and SPL-LV4 strains) and *Ca. P. australiense* were used as references.

RESULTS

Detection of phytoplasmas from papaya using PCR. Of 70 symptomatic samples collected in Northern Mindanao, 8 samples (4 from Natumolan area and 4 from Malitbog area) were positive to phytoplasmas using fu5/rU3 universal primers (Figure 1) but none were positive using group-specific primer Tuf f40/r1150. All symptomless samples were negative to PCR.

RFLP Analysis. RFLP analyses of the 16S rRNA gene using *TaqI* restriction digest enzyme indicated that the phytoplasmas associated with PCY disease were identical to *Ca. Phytoplasma aurantifolia*, the phytoplasma associated with papaya yellow crinkle and papaya mosaic in Australia and not *Ca. P. australiense* causing papaya dieback.

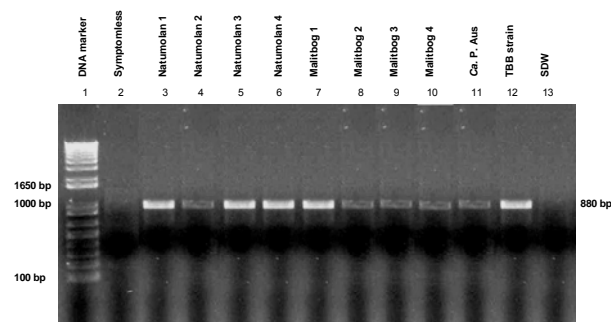


Figure 1. Lane 1, 1 kb plus DNA ladder; 2, symptomless papaya; 3–6, diseased papaya from Natumolan area; 7–10, diseased papaya from Malitbog area; 11, *Ca. P. australiense* (*Ca. P. aus*); 12, *Ca. P. aurantifolia*-TBB strain; 13, sterile distilled water

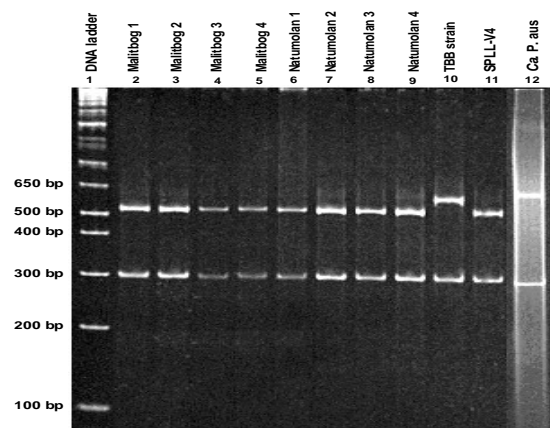


Figure 2. Digestion of fu5/rU3 PCR products with *TaqI* restriction enzyme. Lane 1, 1 kb plus DNA ladder, 2–5, diseased papaya from Malitbog area; 6–9, diseased papaya from Natumolan area, 10, *Ca. P. aurantifolia*-TBB strain; lane 11, *Ca. P. aurantifolia*-SPLL-V4 strain, lane 12, *Ca. P. australiense*.

DISCUSSION.

This is the first report of a phytoplasma association in diseased papaya in the Philippines. The identification of the associated pathogen for PCY is an important step to be able to establish control measures for the disease.

ACKNOWLEDGEMENT

Del Monte Philippines, Inc. for the research fund.

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Phytoplasma diseases in citrus orchards of Pakistan

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INTRODUCTION

Citrus fruits are one of the major export commodities of Pakistan and are grown in an area of 160,000 ha with production of 1.5 MMT annually (1). Most of this citrus is grown in the province of Punjab. *Citrus* species and hybrids [Sapindales: Rutaceae] are affected by a number of destructive diseases and plants infected with '*Candidatus* Phytoplasma asteris' have been detected in orchards close to Islamabad (4). However, the extent of infection is not known and the vector(s) has not been identified. Therefore, a study is under way to detect the presence of phytoplasmas in citrus from orchards in the districts of Sahiwal, Pakpattan and Multan, some 500 km west of Islamabad in the province of Punjab, as well as to identify possible alternative hosts and the vector(s).

MATERIALS AND METHODS

Leaves showing phytoplasma-like symptoms were collected from plants including sweet and blood oranges, grapefruit (*C. × aurantium* L.), mandarins (*C. reticulata* Blanco), lemons (*C. × limon* (L.) Osbeck) and limes (*C. × aurantiifolia* (Christm.) Swingle) [Sapindales: Rutaceae]. In order to identify possible alternative hosts, weeds including couch grass *Cynodon dactylon* (L.) Pers. and wild oat *Avena fatua* L., [Poales: Gramineae], field bindweed *Convolvulus arvensis* L. [Solanales: Convolvulaceae], and fat-hen *Chenopodium album* L. [Caryophyllales: Chenopodiaceae] were collected. Potential vectors, including Asiatic citrus psylla *Diaphorina citri* Kuwayama [Hemiptera: Psyllidae], and leafhoppers (possibly *Balclutha punctata* (Fabricius) and *Empoasca decipiens* Paoli [Hemiptera: Cicadellidae]) were collected, most from around plants showing phytoplasma-like disease symptoms.

DNA was extracted from the petioles and midribs of samples using the method of Doyle and Doyle (3). Both single and nested PCR were used to amplify phytoplasma DNA sequences. Single PCR used the O-MLO primers of Doyle and Doyle (3). The P1/P7 primer pair of Deng and Hiruki (2) and Schneider et al (6) was used in conjunction with primers R16F2n/R16R2 and R16mF2/R16mR1 (5) for nested PCR. DNA of '*Candidatus* Phytoplasma aurantifolia', obtained from Central Science Laboratory, UK, was used as a positive control during amplifications.

RESULTS

Single PCR amplified a 558 bp sequence from the phytoplasma 16S rRNA gene of from DNA extracted from infected plants and nested PCR amplified a 1.2 kb fragment confirming infection with a phytoplasma (Fig. 1). The amplicons will be sequenced to determine which group the phytoplasma belongs to. To date, a total of 20 samples of sweet orange have been tested from the Sahiwal district, 15 from farmer orchards and 5 from the Horticulture Research Center at Sahiwal: 6 samples from farmers' orchards and 3 from the Center were found to be infected. Screening of the insects as well as the weeds collected from the orchards is in progress and the survey is currently being extended to include the rest of Punjab Province to ascertain the incidence and prevalence of disease caused by phytoplasmas.

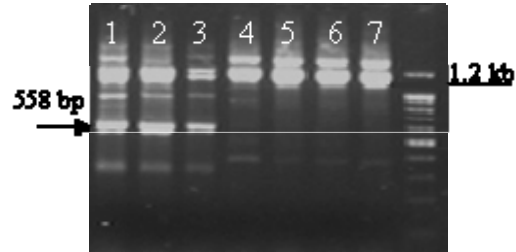


Figure 1. Typical PCR amplification products. Lanes 1–3, single PCR; lanes 4–7, nested PCR; lanes 1, 2 and 4–6, infected sweet orange; lanes 3 and 7, control ('*Ca.* Phytoplasma aurantifolia'); lane 8 molecular weight markers

ACKNOWLEDGEMENT

We would like to acknowledge the Higher Education Commission of Pakistan for their financial support of this study.

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Mechanisms modulating fungal attack in postharvest pathogen interactions and their modulation for improved disease control

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As biotrophs, insidious fungal infections of postharvest pathogens remain quiescent during fruit growth while at a particular phase during ripening and senescence the pathogens transform to necrotrophs causing typical decay symptoms. Exposure of unripe hosts to pathogens (hemi-biotroph or necrotrophs), initiates defensive signal-transduction cascades that limit fungal growth and development. Exposure to the same pathogens during ripening and storage activates a substantially different signalling cascade which facilitates fungal colonisation. This presentation will focus on modulation of postharvest host-pathogen interactions by pH and the consequences of these changes. Host pH can be raised or lowered in response to host signals, including alkalinisation by ammonification of the host tissue as observed in *Colletotrichum* and *Alternaria*, or acidification by secretion of organic acids as observed in *Penicillium* and *Botrytis*. These changes sensitise the host and activate transcription and secretion of fungal hydrolases that promote maceration of the host tissue. This sensitisation is further enhanced at various stages by accumulation of fungal ROS that can further weaken host tissue and amplifies fungal development. Several particular examples of coordinated responses which follow this scheme in *Colletotrichum* and *Penicillium* will be described, followed by discussion of the means to exploit these mechanisms for establishment of new approaches for postharvest disease control.

ABA-dependant signalling of PR genes and potential involvement in the defence of lentil to *Ascochyta lentis*

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INTRODUCTION

Pathogenesis-related (PR) proteins are an important component of inducible defence mechanisms in plants. Their accumulation is triggered by pathogen attack or by abiotic stress (1). Recently, a PR-4 and a PR-10a gene were differentially transcribed in response to the important fungal pathogen, *Ascochyta lentis*, between the resistant (ILL7537) and susceptible (ILL6002) lentil genotypes (2).

This paper outlines the cloning and expression analyses of the PR-4 and PR-10a genes from ILL7537 in response to exogenous treatments of the global signalling molecules abscisic acid (ABA), the immediate ethylene precursor aminocyclopropane carboxylic acid (ACC), methyl jasmonate (MeJA) and salicylic acid (SA); known to control plant defence pathways (3). This will give an insight into the regulatory mechanism controlling specific PR gene expression in lentil and if broadly applicable to defence, these genes may be targeted for future resistance breeding strategies.

MATERIALS AND METHODS

SMART RACE cDNA amplification (Clontech, USA) enabled full-length cDNA cloning of the lentil PR-4 and PR-10a cDNAs. For expression studies, 14-day-old seedlings were sprayed with a 100 μ M solution of ABA, ACC, MeJA or SA. Seedlings were also inoculated with a 10^5 *A. lentis* spore suspension. Control plants were sprayed with sterile water. Bulk seedling foliage (leaf and shoot) from five plants was harvested at 6, 24, 48, and 96 hours post treatment (hpt) using the RNeasy Plant Mini Kit (Qiagen, USA). The bioassay was repeated with another set of independently grown seedlings. cDNA synthesis was carried out by reverse-transcribing 1.5 μ g of each RNA sample using an oligo dT18 primer (Roche, Germany) and the Omniscript RT kit (Qiagen, USA). Triplicate qPCR reactions were performed on each hpt cDNA sample. All PCR products were subjected to melting curve analysis and the comparative C_t method ($\Delta\Delta C_t$) was used to calculate the relative fold changes of gene expression.

RESULTS

The full length PR-10a cDNA was 783 bp long with an ORF encoding a peptide of 156 amino acids with an N-terminal methionine and a C-terminal leucine. The full length PR-4 cDNA was 636 bp long, with 39 bases of 5' untranslated and 157 bases of 3' untranslated sequence, and a poly(A) tail.

PR-4 and PR-10a gene expression was up-regulated in lentil by ABA at all hpt with PR-10a being more highly expressed than PR-4. Neither gene was up-regulated by the other signalling molecules (ACC, MeJA and SA; Fig 1). Thus we proposed that the signalling pathway for both of these genes is ABA-dependent and JA-independent.

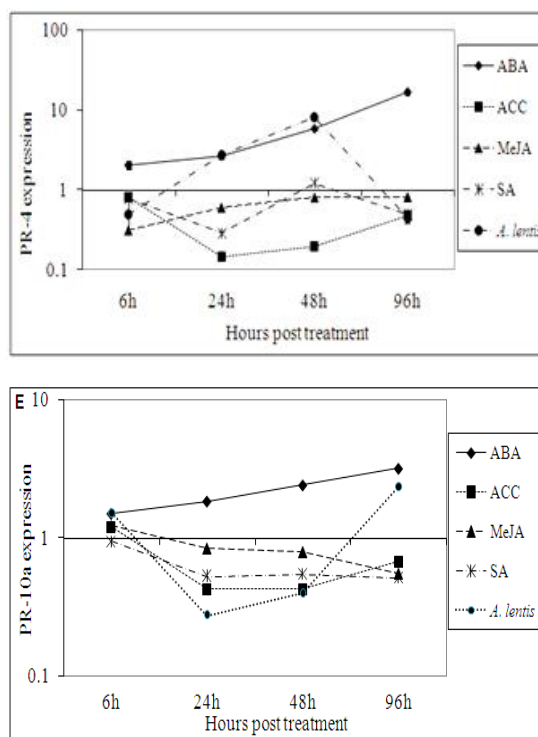


Figure 1. Relative fold changes in transcript levels of PR-4 and PR-10a in 14-day-old ILL7537 seedlings at 6, 24, 48 and 96 hpt following ABA, ACC, MeJA, SA or *Ascochyta lentis* treatment.

DISCUSSION

Since, both PR-4 and PR-10a were also up-regulated in response to the important fungal pathogen *Ascochyta lentis* (2), we propose that ABA may play a pivotal role in the signal transduction of defence responses against *A. lentis* in lentil. This is in agreement with other host/pathogen interaction studies (4). However, further functional validation of ABA modulation of defence responses to such pathogenic stimuli is required to facilitate the identification and characterisation of key genes involved in the ABA-dependent signalling pathway in lentil.

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Fundamental components of resistance to *Phytophthora cinnamomi*: using model system approaches

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INTRODUCTION

Compared with the number of plant species known to be susceptible to *Phytophthora cinnamomi*, few are known to be resistant (1). Understanding how plants are able to resist this pathogen will enable strategies to be developed to enhance individual species survival and to restore structure and biodiversity to the ecosystems under threat. Natural resistance to pathogens can be characterised at a number of levels ranging from specific gene involvement to whole plant responses.

The aims of this project are to determine at the cellular, biochemical and molecular levels what constitutes resistance of native plants to *P. cinnamomi* by identifying the pathways within plants that regulate resistance and then exploring the potential to manipulate them. Our research is centred on further defining and understanding natural resistance in native Australian plant species. Most studies on resistance against pathogens have been undertaken on aerial plant parts (ie stems and leaves) thus there is a requirement for a good, fundamental understanding of root-based resistance. Here we have used a model system approach to characterise the fundamental components of resistance against *P. cinnamomi*.

MATERIALS AND METHODS

Arabidopsis model. The interaction between *Arabidopsis thaliana* ecotype Columbia-0 and *P. cinnamomi* was examined in detail (2). Leaf and root analysis and examination of responses in signal transduction pathway mutants were carried out.

Zea model. Gene expression analysis of defence-related genes was conducted following inoculation of *Zea mays* root tissue over several time points ranging from 0–120 hours. In addition, regulation of defence-related genes in response to the defence hormones jasmonic acid, salicylic acid and ethylene was examined to determine which signalling pathways may operate in this pathosystem.

Lupin model *L. angustifolius* was grown in a soil-free plant growth system (3) and roots inoculated with zoospores. This well recognised, highly susceptible interaction is typified by the development of dark lesions in the root that extend through the vascular system causing root decay. We developed a non-targeted method to extract, separate and identify metabolites produced following inoculation.

RESULTS

Arabidopsis thaliana. *P. cinnamomi* was found to induce active defence responses in Arabidopsis. Tissue specific differences in levels of infection and defence responses induced were found between inoculated root and leaf tissue. Molecular analysis of defence-related genes also showed differential induction between root and leaf tissue. It is likely that the resistance/tolerance that Arabidopsis displays against *P. cinnamomi* is provided by a multi-faceted defence response.

Zea mays. A resistant monocot model system (*Zea mays*) for gene expression analysis of defence pathways was optimised. A suite of maize defence-related genes was analysed in response to infection with *P. cinnamomi*. Genome-wide analysis using a

maize specific microarray has identified genetic pathways that regulate defence to *P. cinnamomi*.

Lupinus angustifolius. The metabolic profiles (in the form of HPLC chromatograms) from both un-inoculated (healthy) and inoculated (diseased) plant root tissue were compared. The data strongly suggests differences in metabolic activity. Multiple experimental repeats were performed and were statistically analysed using principal components analysis which confirmed that the profiles were statistically different. The variables loading identified the peaks/metabolites that caused the data to separate into distinct groups. The separated extracts were then passed through a Time of Flight Mass Spectrometer (TOF MS/MS) to determine identity/chemical structure of metabolites.

DISCUSSION

We have examined the potential for using model plant systems for the analysis of the interaction of *P. cinnamomi* with roots. This research has provided a number of important outcomes that will alter the way in which we approach disease caused by *P. cinnamomi* in Australian native plants. If we know what the mechanisms of resistance are then there is the potential to manipulate them and to devise new methods for control that may include induction of specific resistance mechanisms in susceptible species and development of markers for resistance (for example, anatomical, morphological, molecular-based). Additionally, we are developing screening techniques that will enable concentration of effort on those species that are the most susceptible, vulnerable and rare. Resistant plants can also be used to restore vegetation structure on *P. cinnamomi*-affected sites where the pathogen may still be present.

ACKNOWLEDGEMENTS

We thank the Australian Government Department of Environment, Heritage, Water and the Arts for funding. Dr Xavier Conlan and Professors Neil Barnett and Mike Adams (Deakin University and RMIT University) have provided valuable advice on chemical analyses.

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Genes involved in hypersensitive cell death responses during *Fusarium* crown rot infection in wheat

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INTRODUCTION

Hypersensitive plant cell death is activated by the accumulation of hydrogen peroxide and nitric oxide (1), and is strictly controlled by several genes including cysteine proteases, hydrogen peroxide and superoxide scavengers, and cell death regulators (2). In contrast to biotrophic fungal pathogens, necrotrophic pathogens like *Fusarium pseudograminearum* and *F. culmorum* that cause *Fusarium* crown rot infections, benefit from plant cell death by utilising dying plant tissue to facilitate their spread throughout the plant (3).

MATERIALS AND METHODS

Seedling Germination.—Seeds of the *Fusarium* crown rot susceptible wheat cultivar Puseas and partially resistant wheat line 2–49 were sterilised in 5% NaOCl for 1 hour and were then germinated in the dark on petri dishes containing 2% water agar.

Seedling Inoculation.—Seedlings were inoculated with a single spore of *F. culmorum* or *F. pseudograminearum* on a 2% water agar block using an adapted procedure of Mergoum *et al.* (4) and harvested 10 days post-inoculation.

Microarray analysis of *F. culmorum* infection. RNA was extracted from non-inoculated and *F. culmorum* inoculated seedlings of 2–49 and Puseas and hybridised to Affymetrix® wheat gene chips. Gene transcripts in the inoculated treatments determined to be significantly induced or repressed two-fold over the non-inoculated treatments were analysed using the GeneSpring GX_7_3 program (Agilent).

Deoxynivalenol (DON) Application.—10 ul of 10 mg/ml deoxynivalenol was applied to a block of 2% water agar attached to growing seedlings of 2–49 and Puseas and was taken up by the seedling for 24 hours.

Staining for cell death was visualised in some of the seedlings by applying a second agar block containing 10 ul of 0.1% Evans blue dye below the block containing DON and allowing the stain to be taken up with the DON for 24 hours.

RNA extraction, cDNA, and real-time PCR.—RNA from *F. pseudograminearum* inoculated or DON applied seedlings was extracted using the Plant RNA Purification Reagent protocol (Invitrogen). cDNA was produced using gene specific primers in a reverse transcriptase reaction. cDNA transcripts were assayed using real-time quantitative PCR using SYBR green in the Rotor-Gene 6000 thermocycler.

RESULTS AND DISCUSSION

Table 1. Gene transcript levels expressed during infection with *F. culmorum*.

Genes	2–49	Puseas
Cathepsin B	1.58	2.09
Mlo-like protein	2.26	-1.05
Catalase	-2.19	-4.40
Manganese SOD	40.69	1
superoxide dismutase **		

Genes involved in the hypersensitive cell death response during *F. culmorum* infection (Table 1) were identified using a microarray analysis with the Affymetrix® wheat chip. Cathepsin B, a plant cysteine protease, was induced in the susceptible cultivar Puseas during *F. culmorum* infection. Catalase, an enzyme preventing hydrogen peroxide accumulation, was repressed in Puseas. A Mlo-like protein (cell death regulator) and manganese superoxide dismutase were up-regulated in the resistant wheat line 2–49. These genes are under current investigation during infection studies with *F. pseudograminearum* and DON application to determine what role they have in the response of these cultivars to infection.

Infection with *F. pseudograminearum* spores and DON has been shown to elicit hydrogen peroxide formation and plant cell death as well induce genes involved in defence responses in wheat (5). It is not known whether hypersensitive cell death or avoidance of hypersensitive cell death during infection with *Fusarium* species plays a role in the susceptibility or resistance of wheat cultivars to *Fusarium* crown infection. Further investigation of these genes during the infection process with *F. pseudograminearum* and DON is needed in order to determine if different levels influence hypersensitive cell death and the role they have in either enhancing susceptibility or resistance in wheat during *Fusarium* crown infection.

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Fishing for *Phytophthora* across Western Australia's water bodies

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INTRODUCTION

Most *Phytophthora* surveys in native ecosystems in Australia have focused exclusively on isolations from samples of soil and symptomatic plant tissue including the extensive vegetation health surveys conducted in Western Australia (WA) (1). The outbreak of *P. ramorum* in California and Europe, where early detection of an infested area was important to the success of containment and eradication efforts, has popularised the stream surveys in native ecosystems. In Australia, it has recently been used to detect *Phytophthora* spp. in Victoria resulting in several species being isolated from four streams which varied according to the winter and summer sampling season (2). In our study, the baiting technique was used to survey a wide range of WA's waterways for *Phytophthora* spp. during October to early December 2008.



Figure 1. Water bodies in nine regions of Western Australia sampled for *Phytophthora* species in October to December 2008. Some sites were sampled on four occasions. Region 6 is Perth.

MATERIALS AND METHODS

Waterways (streams, lakes, ponds and estuaries) in WA were sampled up to four times during October to early December 2008. Sites were selected across 50 regional locations from Kununurra (northern site) to Esperance (southern site), and 37 locations in the Perth suburbs (Figure 1, Table 1). Bait bags containing leaves of *Banksia attenuata*, *Pittosporum* sp., *Hakea* sp. and *Quercus* sp., and lupin seedlings were sent via overnight post to 16 volunteers who deployed, and retrieved and returned baits after ~10 days in the water. Leaves were plated onto NARPH agar plates, a medium selective for *Phytophthora*, and incubated in darkness for up to 2 weeks at 20°C during which plates were periodically checked for *Phytophthora* colonies. Colonies were isolated into pure culture and grouped into morpho-types. One representative morpho-type from each site per sampling was identified using the sequence of the ITS region of the rDNA, conducting a BLAST search on Genbank and a phylogenetic analysis (1).

RESULTS AND DISCUSSION

A total of eight *Phytophthora* species were isolated during the October to December 2008 survey of water bodies in nine regions of WA (Table 1). Species yet undescribed were assigned taxa numbers as described in (1). The most frequently isolated species in the southwest were P.sp.12 (VHS5185), P.sp.13

(VHS16108) and *P. inundata*. That *P. inundata* is apparently widespread in the south coast and some wheatbelt regions is of concern given that it has been associated with dying native vegetation in WA's southwest (3). Currently, little is known about P.sp.12 and P.sp.13, but our study clearly shows that they are widespread across many regions of WA.

P.sp.3 and P.sp.11 were not common (Table 1); P.sp.3 has previously been isolated from dying *Eucalyptus marginata*, *Banksia* spp. and *Pinus radiata* (1). P.sp.8, P.sp.11, P.sp.12 and P.sp.13 fit within a strongly supported clade with P.sp.3, and (1) proposed that these would be common in water bodies. Our sampling during October to December 2008 shows that this hypothesis is true with the exception of P.sp.3 and P.sp.11.

Table 1. *Phytophthora* species found in water bodies in nine regions of Western Australia during the October to December 2008 sampling (see Figure 1). Reg = Region, na = not applicable (too few samplings)

Reg.	<i>Phytophthora</i> spp.	Most common spp.
1	<i>P. inundata</i> , P.sp.11, P.sp.13	P.sp.13
2	<i>P. inundata</i> , P.sp.11, P.sp.13	<i>P. inundata</i>
3	<i>P. inundata</i> , P.sp.13	<i>P. inundata</i>
4	P.sp.3, P.sp.12, P.sp.13	P.sp.12, P.sp.13
5	P.sp.12	na
6	<i>P. hydropathica</i> , <i>P. inundata</i> , P.sp.3, P.sp.8, P.sp.11, P.sp.12, P.sp.13	P.sp.12
7	P.sp.8, P.sp.11	P.sp.8
8	<i>P. inundata</i> , P.sp.3, P.sp.8, P.sp.11, P.sp.13	<i>P. inundata</i> , P.sp.8
9	<i>P. parvispora</i> , <i>P. hydropathica</i>	na

P. hydropathica frequently isolated from irrigation water, was only isolated on two occasions; once in Perth and once in the north of WA from an irrigation channel (Table 1). Additionally, *P. cinnamomi* var. *parvispora* was only recovered once during the sampling, also from the irrigation channel in the north. Our results in relation to the Victorian study will be discussed.

ACKNOWLEDGEMENTS

We thank all the volunteers who deployed and returned the baits and WWF for funding support. Additional assistance was provided by C. Fletcher, T. Paap, D. White and N. Williams. More information at www.f4p.murdoch.edu.au.

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Incidence of fungi isolated from grape trunks in New Zealand vineyards

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INTRODUCTION

With the growth of the New Zealand wine industry in size and geographic distribution, the number of observations of grape vine trunk disease symptoms has increased. Within the industry, identification of fungi present in trunks of unthrifty vines has been a low priority. Previous studies have often focused on a single genus (1) or regional record of disease (2).

In order to reduce the impact of trunk diseases in New Zealand vineyards, it is important first to establish which fungi are present. This survey is a preliminary investigation of the range and incidence of fungi isolated from trunk wood across a number of vineyard sites in New Zealand.

MATERIALS AND METHODS

Field survey. A survey was conducted on vines from 37 vineyard blocks in the North and South Islands of New Zealand. Core samples were taken from the trunks of five vines at each site, using a MATTSON N° 4333 forestry corer. This device removed a 5-mm core approximately 80 cm up the trunk, passing directly through the wood until the bark was ruptured on the far side. The corer was cleaned between samples with 70% ethanol to prevent cross contamination. The entire core sample was transferred in a sterile tube to the laboratory.

Isolations. Each core was surface sterilised for 30 sec in 70% ethanol, 2 min in 3.5% w/v sodium hypochlorite and 30 sec in 70% ethanol. Samples were cut into 5–10 mm pieces and placed on potato dextrose agar (PDA; DIFCO) amended with 100 µg/mL streptomycin sulphate and 100 µg/mL Penicillin G potassium salts and incubated at 20°C with lights (12 h photoperiod).

Identification. After one week, fungi were identified by morphological features and confirmed by amplifying the internal transcribed spacer regions of the rDNA using the polymerase chain reaction (PCR) primers ITS1-F and ITS-4. PCR products were sequenced using the BigDye Terminator V. 3.1 cycle sequencing kit (Applied Biosystems, UK). The resultant sequences were characterised by Basic Local Alignment Search Tool analysis from the most closely related sequences on GenBank. Fungal morphological characteristics were re-examined after a month to confirm identification further and to allow time for slower growing fungi to be isolated and identified. Not all fungi were identified to the species level so results are given as a summary by genera.

RESULTS

The most commonly isolated fungi were species of the genera *Botryosphaeria*, *Phaeoconiella* and *Eutypa* at multiple sites (Table 1). Less commonly, *Phaeoacremonium* spp. and *Cylindrocarpon* spp. isolates were also found. The same genera were not isolated from all 37 sites sampled. *Phaeoacremonium* sp. were found only in Hawke's Bay, although the other fungal isolates were not confined to a single region. Other fungi isolated during the survey included species of *Acremonium*, *Alternaria*, *Cadophora*, *Cladosporium*, *Epicoccum*, *Gliocladium*, *Mucor*, *Penicillium*, *Phoma*, *Trichoderma*, *Ulocladium* and *Xylaria*.

Table 1. Recoveries of fungal genera regarded as wood pathogens from a survey of thirty-seven vineyard blocks. The survey of incidence of fungi was conducted in the North and South Islands of New Zealand during 2007 and 2008.

Genus	North Is. n=24	South Is. n=13
<i>Botryosphaeria</i>	20	5
<i>Phaeoconiella</i>	19	3
<i>Phaeoacremonium</i>	3	0
<i>Phomopsis</i>	8	3
<i>Cylindrocarpon</i>	2	3
<i>Eutypa</i>	12	8

DISCUSSION

The incidence of species of *Botryosphaeria*, *Phaeoconiella* and *Eutypa* in many of the vineyard blocks surveyed suggests that these fungi should be the focus of more detailed research to establish their importance to the industry. The isolation of a fungus from a block does not prove that it is the organism responsible for the disease symptoms. If multiple fungi are present, the incidence does not allow the determination of which fungi are the most likely to be causing symptoms at that site.

As the sample size in each vineyard block was limited, we cannot be certain that failure to detect a particular species indicates that these fungi were not present. For example additional isolations will be needed to determine if *Phaeoacremonium* is restricted to Hawke's Bay only.

ACKNOWLEDGEMENTS

Funding for this project was provided by the Ministry of Agriculture and Forestry Sustainable Farming Fund and the Marlborough Wine Research Centre Trust. We would also like to thank all industry representatives who allowed us to sample vines.

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Isolation and characterisation of strains of *Pseudomonas syringae* from waterways of the central North Island of New Zealand

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INTRODUCTION

Epiphytotic of plant bacterial diseases can occur where previously no or little inoculum was thought to be present. The source of the primary inoculum of these epiphytotic is not always easy to determine, especially for pathogenic bacteria, which are good epiphytes, such as *Pseudomonas syringae*, one of the most economically important bacterial plant pathogens. This project aimed to determine whether rivers from the Central North Island of New Zealand could constitute a reservoir for *P. syringae*. *P. syringae* is a complex group of strains which can be grouped in about 50 different pathovars (strains which have the same pathogenicity and the same host range) or in nine genomospecies (strains which belong to the same species based on DNA/DNA homology) (1). In this study we isolated some strains of *P. syringae* and tried to determine to which genomospecies or which pathovar they belong based on polymerase chain reaction (PCR) experiments.

METHODS AND RESULTS

Collection of water samples and isolation of bacteria. Water samples were collected from the Waikato River (Hamilton) and from the Whakapapanui Stream (Tongariro National Park). The isolation of bacteria was carried out as described previously (4). Ten strains from Whakapapanui and five strains from the Waikato River, which showed all the characteristics of strains of *P. syringae*: ability to produce a fluorescent pigment on a modified King's B medium, ability to cause a hypersensitive reaction when infiltrated into tobacco plant, absence of a cytochrome c oxidase and inability to utilise arginine, were retained for further characterisation.

Characterisation by Polymerase Chain Reaction (PCR). All PCR experiments were carried out on an Eppendorf Mastercycler® Gradient using 20 ng of total DNA per reaction. The final reaction volume was 30 µl including 10 µM of each primers and 1 unit of *i-Taq*™ from INtRON Biotechnology Inc. The primers and the programs were those published earlier (e.g. 2). For each experiment, a negative control, in which the DNA solution was replaced by water, and a positive control, in which the DNA was that of a strain we knew would give a positive reaction, were used. Of the 15 strains analysed, five gave a positive reaction with primers specific to strains of genomospecies 1, which is represented by *P. syringae* pv. *syringae*. None gave a positive response with primers specific for strains of genomospecies 2, which is represented by *P. syringae* pv. *phaseolicola* and *P. syringae* pv. *morsprunorum*. None gave a positive response when PCR protocols specific for *P. syringae* pv. *papulans*, *P. syringae* pv. *tagetis*, *P. syringae* pv. *helianthi* or *P. syringae* pv. *actinidiae* were used.

DISCUSSION

Strains of *P. syringae* were isolated from two different water systems: the Waikato River, a complex system which includes lakes and goes through some cultivated and non cultivated lands, and the Whakapapanui Stream, which is fed by melt water from Mount Ruapehu and does not cross cultivated lands. These results and similar ones presented earlier (3) support the hypothesis that the life history of *P. syringae* is linked to the

water cycle, as proposed by Morris et al. (3). In this scenario, rain and melt water containing cells of *P. syringae* feed streams and rivers that bring those cells of *P. syringae* in contact with wild and cultivated plants. The subsequent multiplication of these bacteria as pathogens or epiphytes provides a huge inoculum, part of which might form aerosols that can be taken up by clouds. The ability of some of these bacteria to induce ice nucleation might help the formation of rain and/or snow and explain the presence of these bacteria in rain and snow. Although strains of *P. syringae* have been found in a river and a stream fed by melt water, to complete the cycle we need to demonstrate that those same strains are also found on or in plants as epiphytes or as pathogens. The characterisation of the strains of *P. syringae* isolated from New Zealand waterways would allow this demonstration. The characterisation is continuing, with more strains being analysed including strains being isolated from different waterways, and with more techniques being utilised including molecular techniques different from PCR.

ACKNOWLEDGEMENTS

We thank The New Zealand Institute for Plant and Food Research Limited for their support.

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Evaluation of fungicides to manage brassica stem canker

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INTRODUCTION

Leptosphaeria maculans and *Rhizoctonia solani* AG 2.1, 2.2 and 4 are the dominant soil borne fungal pathogens causing Brassica stem canker (1). Brassica stem canker causes stem rot, resulting in plant collapse before harvest or stem breakage during harvest. Greenhouse trials were undertaken to evaluate pre-planting and post-planting fungicide drenches for the control of *R. solani* AG 2.1, 2.2 and 4 and *L. maculans*.

MATERIALS AND METHODS

R. solani. Coco peat potting mix was inoculated with either AG 2.1, 2.2 or 4 by mixing in a 1L slurry of 12 macerated plates of 5–7 day old cultures grown on potato dextrose agar (PDA). The inoculated mix was placed into MK12 pots and incubated in the greenhouse at 22°C for 7 days to allow the *Rhizoctonia* to establish in the soil.

Six-week-old susceptible cauliflower speedlings (cv. Chaser) (2) were immersed in a fungicide mix (Table 1) for 5 mins to ensure the fungicide had permeated the soil and root matrix before planting into the inoculated soil mix. Ten replicate plants were used per treatment, with a water drench used as the control treatment.

Plants were maintained in a greenhouse at 22°C and assessed weekly for stem canker using a disease severity rating scale of 0–100 where; 0 = healthy, 20 = superficial staining, 40 = canker girdling ½ stem, 60 = canker girdling full stem, 80 = severe canker (wilt) and 100 = plant death. The presence of *R. solani* in the pots was confirmed 4–6 weeks after planting by baiting with toothpicks, whereby toothpicks were placed in the pots for 24 hrs, washed and incubated on PDA (3).

L. maculans. Four-week-old cauliflower seedlings cv. Chaser were planted into MK12 pots with coco peat. Six replicate plants were treated with 30 ml each of fungicide drench (Table 2) applied to the soil surface either two days before or two days after mycelial plug inoculation (2). Plants were maintained and assessed similarly to the *R. solani* trial.

RESULTS AND DISCUSSION

R. solani. All controls were infected with *R. solani*, AG 2.1 being the most virulent and AG 2.2 the least virulent, with 100% and 70% of untreated plants infected respectively. None of the fungicides effectively controlled the disease; however Amistar, Cabrio, Maxim, Sumisclex and Jockey did reduce the severity (Table 1). The different AG groups responded differently to the fungicides, for example Rizolex at 0.4ml/L did not suppress AG 2.1 or 4, but was effective against AG 2.2. *R. solani* was detected in soil from all treatments (data not presented).

L. maculans. The disease developed slowly, with symptoms not showing on many plants until 10 weeks after inoculation. All control plants were infected, and none of the treatments were effective when applied after inoculation (Table 2). Maxim, Cabrio and Rovral provided some suppression of disease when applied before inoculation, and complete control was achieved with a pre-plant drench of the higher rate of Amistar.

Table 1. Mean per cent severity of stem canker symptoms on plants 8 weeks after being drenched with a fungicide prior to planting into *R. solani* inoculated soil.

Treatment	Rate /L	AG2.1	AG2.2	AG4
Untreated		74	24	46
Sumisclex 500	0.75ml	18	12	18
Rizolex liquid	0.2ml	78	14	42
Rizolex liquid	0.4ml	62	2	82
Jockey	1ml	10	6	26
Terrachlor	2g	52	16	24
Rovral Aquaflo	0.5ml	36	6	36
Rovral Aquaflo	1ml	30	6	34
Amistar	0.5ml	20	4	10
Amistar	1ml	16	10	14
Cabrio	0.4ml	16	12	22
Maxim	0.4ml	16	6	10
L.S.D (P=0.05)		14.5	12.7	17.0

Table 2. Per cent incidence (inc) and severity (sev) of stem canker symptoms on plants drenched with fungicides and inoculated with *L. maculans* before or after treatment.

Treatment	Rate /L	Pre inoc. drench		Post inoc. drench	
		Inc	Sev	Inc	Sev
Untreated		100	70	100	27
Sumisclex 500	0.75ml	100	40	100	67
Rizolex liquid	0.2ml	67	17	83	27
Rizolex liquid	0.4ml	83	30	100	27
Jockey	1ml	83	27	100	24
Terrachlor	2g	100	20	100	30
Rovral Aquaflo	0.5ml	83	17	100	44
Rovral Aquaflo	1ml	17	3	100	23
Amistar	0.5ml	50	14	67	24
Amistar	1ml	0	0	67	20
Cabrio	0.4ml	33	7	100	27
Maxim	0.4ml	33	7	67	20
LSD (P=0.05)		-	16.2	-	16.5

CONCLUSION

No fungicide treatments were effective in controlling stem canker; however suppression was achieved with a pre planting drench of either Amistar, Cabrio or Maxim.

ACKNOWLEDGEMENTS

This project was facilitated by HAL in partnership with AUSVEG and was funded by the National Vegetable Levy. The Australian Government provides matched funding for all HAL's R&D activities.

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Evaluation of spray programs for powdery mildew management in greenhouse cucumbers

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INTRODUCTION

Powdery mildew (*Podosphaera xanthii*) is a serious disease of greenhouse cucumbers worldwide causing defoliation and premature senescence of crops. The disease is mainly managed with fungicides and management is becoming increasingly difficult due to fungicide resistance and the lack of registered products for greenhouse use. Trials were conducted to examine spray intervals for conventional fungicides and to determine whether 'soft' products could be effectively incorporated into spray programs for powdery mildew.

MATERIALS AND METHODS

Spray program trials were conducted on cucumber plants in a research greenhouse. In the first trial various fungicide programs were compared at spray intervals of 7 or 14 days (Table 1). The second evaluated programs incorporating 'soft' products that were effective in preliminary screening trials (Table 2). Two week old seedlings were exposed to infected plants (Day 0) and exposure continued for the entire trial. Sprays commenced at day 14, with 10 plants per treatment. Severity of powdery mildew was assessed on leaves on day 0 and then every 7 days on sprayed leaves. Relative Area Under Disease Progression Curves (RAUDPC) (1) for each spray program were analysed with ANOVA (Statistix 8).

RESULTS AND DISCUSSION

All 7 day interval programs reduced the severity of powdery mildew significantly more than the 14 day programs. Although the program with Bayfidan® and Cabrio® at 14 day intervals had significantly more disease than the 7 day programs, the disease level may still be commercially acceptable (Figure 1; Table 1).

Programs incorporating the 'soft' products Ecocarb® and Rezist® with Amistar®, and the Morestan®/BioCover® program were as effective as Morestan®/Bayfidan®. Bayfidan®/Rezist® was the least effective treatment (Figure 2; Table 2).

Table 1. Spray schedules and Relative Area Under Disease Progress Curves (RAUDPC) for trial examining spray intervals in conventional programs.

Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	RAUDPC
M	M	M	B	B	B	0.70 a
M	M	C	C	B	B	1.11 a
B	B	B	C	C	C	1.19 a
C	C	C	B	B	B	1.39 a
M	-	M	-	B	-	2.76 b
C	-	C	-	B	-	6.43 c
M	-	C	-	B	-	7.93 d
B	-	B	-	C	-	8.18 d
Untreated control						33.9 e

M=Morestan® (250g/kg oxythioquinox) 0.4g/L; B=Bayfidan® 250 EC (250g/L triadimenol) 0.4mL/L; C=Cabrio® (250g/L pyraclostrobin) 0.4mL/L. Means with the same letter are not significantly different (P<0.05).

Table 2. Spray schedules and Relative Area Under Disease Progress Curves (RAUDPC) for trial examining 'soft' products in conventional spray programs.

Day 14	Day 28	Day 42	Day 56	RAUDPC
A	R	A	R	4.26 a
M	M	Bi	Bi	5.15 ab
E	A	E	A	6.57 ab
M	M	B	B	6.92 b
B	A	B	A	7.39 bc
B	R	B	R	9.74 c
Untreated control				21.98 d

A=Amistar® 250SC (250g/L azoxystrobin) 0.8mL/L; M=Morestan® (250g/kg oxythioquinox) 0.4g/L; B=Bayfidan® 250EC (250g/L triadimenol) 0.4mL/L; Bi=BioCover® (840g/L petroleum oil) 10mL/L; E=Ecocarb® (940g/kg potassium bicarbonate) 4g/L +Synretrol®HortiOil (905g/L emulsifiable botanical oil) 2.5mL/L; R=Rezist® 1.5mL/L+Sett Enhanced 2mL/L. Means with the same letter are not significantly different (P<0.05).

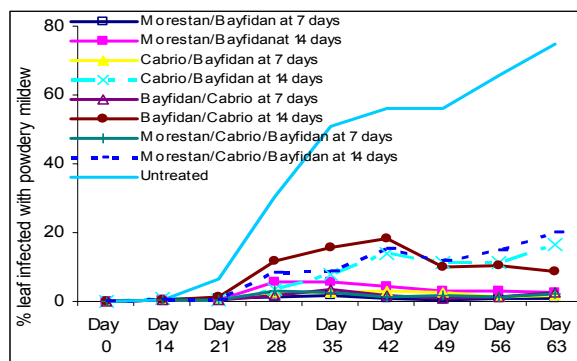


Figure 1. Disease progress curve for Trial 1.

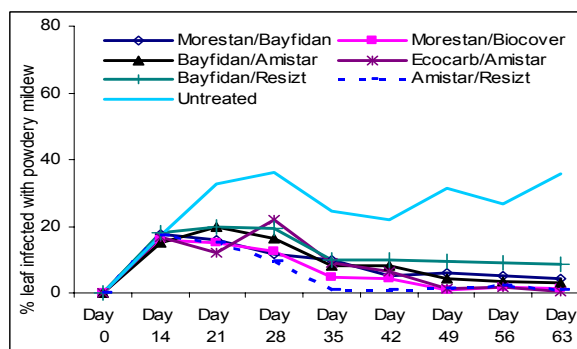


Figure 2. Disease progress curve for Trial 2.

Results indicated that 'soft' products could be incorporated effectively into spray programs.

ACKNOWLEDGEMENTS

This project was facilitated by HAL in partnership with AUSVEG and was funded by the National Vegetable Levy. The Australian Government provides matched funding for all HAL's R&D activities.

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The incidence of copper tolerant bacteria In Australian pome and stone fruit orchards

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INTRODUCTION

Australian pome and stone fruit production relies heavily on copper-based fungicides for control of bacterial diseases including bacterial blast and bacterial canker (*Pseudomonas syringae*). Copper fungicides have had a long history of use for control of fungal diseases and general clean up during the dormant period. The impact of their long term application on development of copper tolerant bacterial populations in fruit orchard is not known. The authors had initiated collection and screening of copper tolerant bacteria from Australian pome and stonefruit fruit orchards since 2007. This study presents an analysis of the copper tolerant (CuT) bacterial populations detected in the 2008–2009 growing season.

MATERIALS AND METHODS

Pome and stone fruit blossoms were collected during the spring of 2008, from major fruit growing regions in Victoria, New South Wales, Queensland, Tasmania and South Australia. Up to four samples consisting of ten blossoms, one from each tree, were collected from monitored orchard block. Each sample was washed in 5 ml sterile distilled water and a 0.1 ml aliquot was spread on *Pseudomonas* Fluorescent Agar (PFA, Difco). Pure cultures were obtained by repeated streaking and dilution plating. The purified bacteria were characterised based on cultural and biochemical properties: namely, production of fluorescent pigments, hypersensitivity reaction on tobacco, and oxidase, levan and arginine reactions (1,2). Whilst additional tests are being conducted to further identify the bacteria, for the purpose of this report, they were categorised as fluorescent *Pseudomonas* spp., yellow bacteria, and non-fluorescent, non-yellow (NFY) bacteria.

The sensitivity of bacteria to copper ions was tested using a ten fold dilution of a slightly turbid bacterial suspension, yielding 10^6 – 10^8 colony forming units, determined by dilution plating on PFA. Four 10 μ l drops of each bacterial suspension were added to casitone-yeast-extract medium (3) amended with 0, 0.16, 0.32, 0.48 and 0.64 millimolar (mM) of copper sulphate (Cu). Formation of bacterial colony was assessed after incubation at $27 \pm 1^\circ\text{C}$ for 3 days.

RESULTS

A total of 315 isolates comprising 155, 77 and 83 *Pseudomonas*, spp., yellow bacteria, and NFY bacteria respectively were tested for sensitivity to Cu ions (Table 1).

The number of isolates that were able to form confluent colony at 0.32 mM Cu (CuT), the threshold concentration for tolerance to copper ions (4), are presented in Table 1.

Overall, 47.6% of the isolates tested were CuT. Twenty-seven CuT isolates were potentially plant pathogenic based on their ability to induce hypersensitivity (HR+) response in tobacco. Of 155 isolates from apple and pear hosts, 40.0% were CuT, but only 10 isolates i.e. 6.5%, were both CuT and HR+. Six *Pseudomonas* spp. from pome fruit were CuT and HR+, but fourteen CuT and HR+ isolates were obtained from stone fruit hosts. Whilst more than half (68.4%) of the *Pseudomonas* spp. from both pome and stone fruits were CuT, only 14.3 and 39.8% of yellow and NFY bacteria respectively were CuT.

Table 1. The incidence of copper tolerant bacteria in pome and stone fruit orchards in the 2008–2009 season.

Source	<i>Pseudomonas</i> spp.		Yellow bacteria		NFY bacteria	
	Tot	CuT	Tot	CuT	Tot	CuT
Apple and pear	68	44	36	5	51	16
Stone fruit	87	65	41	6	32	17
Total	155	106	77	11	83	33

NFY—Non-fluorescent, non-yellow bacteria.

Tot—Total number of bacterial isolates tested.

CuT—Number of copper tolerant bacterial isolates.

DISCUSSION

The findings indicate detection of CuT bacteria in pome and stone fruit orchards for the first time in Australia. The detection of high proportions of CuT bacteria suggests the potential risk of selection pressure associated with application of copper sprays. The implication of this finding on the effectiveness of copper-based sprays, which is the only chemical treatment available for the control of bacterial diseases, needs further research. Further evaluation of the impact of copper sprays on CuT bacterial populations is required to determine the continued efficacy of copper-based bactericides.

ACKNOWLEDGEMENTS

Funding from DPI, Victoria; Horticulture Australia Limited, Apple and Pear Australia Limited, Canned Fruit Industry Council of Australia and Fruit Grower Victoria. Collaborators: A. Steinhäuser (FGT, Tasmania), S. Hetherington, A. Moorey (DPI NSW), P. James (PIRSA, SA), L. Haselgrove (QDPI, Queensland).

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Lessons from the tropics—the unfolding mystery of vascular-streak dieback of cocoa, the importance of genetic diversity, horizontal resistance, and the plight of farmers

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Cocoa in Papua New Guinea and South-East Asia has been devastated by a mysterious dieback disease at least since the rapid expansion of planting in the 1950s. Investigations into the nature of this disease, its control and the ongoing mystery surrounding it will be described as an example of unusual biology likely to be common in the relatively unexplored biology of the wet tropics. The incredible genetic diversity of plant communities and its importance especially in the wet tropics will be introduced in relation to disease control in food crops and selection of resistance to vascular-streak dieback of cocoa. The nature of the durable resistance of cocoa to vascular-streak dieback will be discussed as a tribute to J.E. van der Plank. Recent changes in the nature of the disease found during a current ACIAR project in Sulawesi will be described as an example of the uncertainty of human knowledge in the face of ever-changing biology. Finally, the plight of cocoa farmers facing serious pest and disease problems in the region will be discussed as an example of the poor situation of farmers throughout the world.

Translating research into the field: how it started, how it is practised and how we carry out grape powdery mildew research

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INTRODUCTION

To understand research translation, it is helpful to understand our past efforts to extend knowledge, especially agricultural knowledge, to those who need and benefit from that information. The traditional term to define this process is Extension. Extension as it exists today in the United States has a rich history of nearly 150 years. We will examine the development of the Cooperative Extension Service in the US, especially at the time (mid 19th century) when educational and research institutions were evolving dramatically (1). In that era of change, colleges began to be more egalitarian and outward looking (extension). Extension has survived and grown over the years, but some of the mission fervour had faded. A renewed commitment to extension is now embodied within the latest buzzword of the 21st century: engagement. We will examine how extension and engagement influence research using the example of grapevine powdery mildew. We will consider what drives the process and how we can sustain it. Some final reflections look towards the future and thoughts on the value of translating research to the field.

EXTENSION'S CREATION AND TRANSITION TO ENGAGEMENT

The Genesis of Extension It was during the American Civil War that an innovative approach was taken to permit a broadened reach of higher education. The Morrill Act of 1862 established a federal partnership with states to establish centres of learning for agriculture and the mechanic arts. The act provided tracts of federal land for states to use or sell in order to create "land-grant" colleges. Shortly thereafter (1865) Cornell University was established as New York State's land-grant college. Almost thirty years later (1894) Cornell's first professor of horticulture, Liberty Hyde Bailey, implemented the nation's first extension program, "a plain, earnest, and continuous effort to meet the needs of the people on their own farms and in the localities."

Extension in the 21st Century Fast forward to 2009, and the modern Extension Service remains one of the very unique features of American agriculture. However, it has moved beyond farmers and addresses many aspects of rural and urban life. In an analysis of the future of the land-grant universities, the Kellogg Commission exhorted these institutions to become "engaged universities" (2). By engagement they refer to institutions that have redesigned their teaching, research, and extension and service functions to become even more sympathetically and productively involved with their communities, however community may be defined."

MEETING THE CHALLENGE

So how does this new engagement play out for a university researcher who has no official extension responsibility? For those of us who carry out mission-oriented research we are always asking two questions: What are the challenges facing the industry? How does my research address those challenges? In the context of plant pathology, our first goal is to carry out good science; but our science is always focused on solving problems as we expand our knowledge.

GRAPEVINE POWDERY MILDEW CHALLENGES WE HAVE ADDRESSED

Over the past 30 years with the assistance of colleagues and students we have examined, and in some cases, redefined the role and impact of *Erysiphe necator* on grape, always driven by industry needs (e.g., 3-5):

- The importance of early-season disease management
- The role of cleistothecia in epidemics
- Environment, ascospore release, and infection
- Early season disease spread within vineyards
- Effect of cold nights on mildew development
- Ontogenic resistance in grape berries
- Heterogeneity of berry development and susceptibility
- Diffuse mildew infection on berries and wine quality
- Signals that turn sporulation on and off
- Biological control of powdery mildew by Tydeid mites

CONCLUSIONS

Our research on the biology and epidemiology of grape powdery mildew has had a major impact on grape production in New York and has influenced research and extension programs throughout the US and beyond. The program is not substantially different from many other mission-oriented research programs, but for those who seek to translate research to the field, this is what we have learned:

- Be grounded – understand and appreciate agriculture
- Observe; look at the big picture, but mind the little stuff
- Know your patients and your pathogens
- Become engaged; know your stakeholders and deal with them as peers
- Write proposals that are clear succinct, and interesting
- Collaborate, locally and professionally
- Train the next generation; get them excited about translational research
- Have fun while doing all this!

ACKNOWLEDGEMENTS

For their vital collaborations, I thank David Gadoury and the labs of Wayne Wilcox, Greg Loeb, Thomas Henick-Kling, Lance Cadle-Davidson, and Ian Dry.

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Stem rust race Ug99: international perspectives and implications for Australia

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INTRODUCTION

Stem rust of wheat, caused by *Puccinia graminis* f. sp. *tritici* (Pgt), is one of the most feared plant diseases. It was particularly problematic in early efforts to grow wheat in Australia, being described by McAlpine (1) as "positively injurious". Reports of losses in Australia include £400,000 in 1903, £2 million in 1916, £7 million in 1947, and \$200 to 300 million in 1973 (2). Concerted efforts to control the disease with genetic resistance began with the release of cultivar Eureka in 1938, and have led to a decline in the incidence of the disease and in the frequency of epidemics.

The detection of stem rust race "Ug99" in Uganda in 1999 has had significant implications for the control of stem rust. Referring to "Ug99", Nobel Laureate Dr Norman Borlaug, stated "The prospect of a stem-rust epidemic in wheat in Africa, Asia, and the Americas is real and must be stopped before it causes untold damage and human suffering" (<http://www.sciencenews.org/articles/20050924/food.asp>).

PATHOGENIC VARIABILITY IN AUSTRALIA

Annual pathogenicity surveys of Pgt conducted at the University of Sydney since 1919 have provided a sound basis for rust resistance breeding efforts. These surveys have revealed 3 incursions of 4 exotic Pgt isolates, all of which had significant impacts on wheat production, highlighting the importance of current exotic threats such as Pgt race "Ug99". The surveys have also shown clear evidence of the importance of pathogen aggressiveness, of single step mutation and of somatic hybridisation in overall pathogen population structure (2).

STEM RUST RACE Ug99

Since its first detection in Uganda in 1999, "Ug99" has been detected in Kenya, Ethiopia, Sudan and Yemen, and in 2007 was detected in Iran (3). It carries virulence matching many resistance genes in hexaploid wheat: genes rendered ineffective are *Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr8b*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr11*, *Sr15*, *Sr17*, *Sr21*, *Sr30*, *Sr31*, and *Sr38*; those that remain effective are: *Sr7a*, *Sr13*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, *Sr27*, *Sr28*, *Sr29*, *Sr32*, *Sr33*, *Sr35*, *Sr36*, *Sr37*, *Sr39*, *Sr40* and *Sr44*. Of particular concern is virulence for gene *Sr31*, one of the most widely deployed stem rust resistance genes, which remained effective until the detection of "Ug99". Since its first detection, two presumed mutational derivatives with virulence for *Sr24* (4) and for *Sr36* (5) have been detected in Kenya (4), and a race with identical virulence but lacking virulence for *Sr31* has been detected in South Africa (6).

"Ug99" has had a large impact on a wide range of international wheat germplasm. In response, the Borlaug Global Rust Initiative (BGRI) was launched in Kenya in 2005 and acknowledged the threat of "Ug99" to stable wheat production in eastern Africa, and also recognised the threat posed to many other parts of the world. More recently, an international project, "Durable Rust Resistance in Wheat" was funded by the Bill and Melinda Gates Foundation and is managed by Cornell University. This project has a range of activities that include pre-breeding, rust pathogen surveillance and cultivar deployment.

IMPLICATIONS FOR AUSTRALIA

The Australian Cereal Rust Control Program (ACRCP) provides support to all groups engaged in cereal breeding in Australia, and undertakes research on the pathology and genetics of rust diseases. This strategy has led to a robust understanding of the resistance genes deployed in Australia, and a resulting ability to predict response of Australian germplasm to new rust races such as "Ug99". These predictions have been refined by field testing germplasm in Kenya with the assistance of the Kenyan Agricultural Research Institute from 2005–07. Because *Sr31* has not been used widely in Australia, the greatest impact of "Ug99" on germplasm to date has been due to virulence for *Sr30*, combined virulence for *Sr38* with other genes, and more recently, virulence for *Sr24* and *Sr36*. While virulences for *Sr30*, *Sr36* and *Sr38* have been detected in Australia, virulence for *Sr24* has not. The genes *Sr2*, *Sr12*, *Sr13*, *Sr22* and *Sr26*, effective against "Ug99" and derivatives, are important contributors to the resistance present in current germplasm.

CONCLUDING COMMENTS

Although "Ug99" may never reach our shores, it must be regarded as a serious exotic rust threat. Whilst acknowledging this, it is equally important not to forget other exotic rust threats, including races of endemic cereal rust pathogens, plus the cereal rust diseases that do not occur here (stripe rust of barley, *Puccinia striiformis* f. sp. *hordei*; leaf rust of durum wheat, *Puccinia* sp. Group II Type A; crown rust of barley, *P. coronata* var. *hordei*).

ACKNOWLEDGEMENTS

The Australian Cereal Rust Control Program is supported financially by the Grains Research and Development Corporation, Australia.

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Mitigating crop losses due to stripe rust in Australia: integrating pathogen population dynamics with research and extension programs

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INTRODUCTION

Wheat stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*, *Pst*) was first recorded in Australia in 1979 and became endemic to the eastern Australian wheat zone causing serious losses in the mid eighties (1). Concerted pathology and breeding R&D combined with industry adoption of resistant varieties resulted in minimal losses for nearly 20 years. The first report of stripe rust in Western Australia in 2002 was the result of a foreign pathotype incursion (2). This aggressive pathotype widened its distribution in following years to encompass the entire Australian wheat production zone, and caused serious losses including increased annual fungicide expenditure ranging from \$AUD40–90million (1).

The stripe epidemic in eastern Australia in 2008 was the most intensive in the 30 year history of the disease in Australia. The dynamics of host resistance and pathogen variability gave rise to a situation that required a close connection between extension and research staff in order to maximise the available resources of host resistance and fungicide availability. This paper presents details of the epidemic development and the interplay of variety resistance and pathogen population dynamics during the 2008 season.

MATERIALS AND METHODS

Rust samples collected and forwarded to PBI by co-operators (advisors, farmers, researchers) were assessed for pathotype determination using described methods (3). Results were immediately reported to co-operators by email. The relationship between pathotype and the resistance genes present in commercial wheats provided a basis for predicting expected disease response.

RESULTS AND DISCUSSION

Samples received from various regions of Australia are illustrated in Figure 1. The epidemic began early from presumed green bridge survival sites, developed slowly in winter, and became explosive in spring; the epidemic was largely confined to NSW and Queensland.

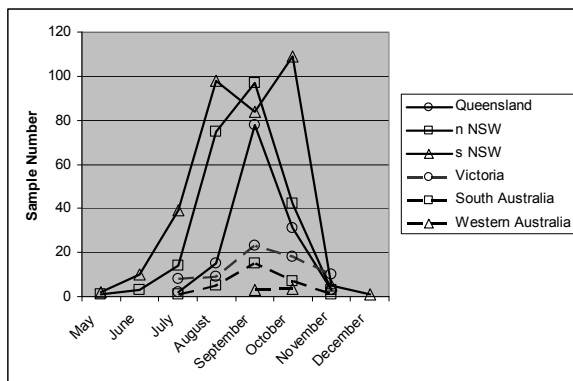


Figure 1. Frequency of *Pst* samples accessioned at PBI Rust Laboratory in 2008 from six regions in Australia.

The major pathotypes detected in the 2008 epidemic are described in Table 1.

Table 1. Features and frequency of the four *Pst* pathotypes detected in Australia in 2008. (R=resistant; S=susceptible)

Pathotype	First Report	2008 n=830	Resistance Gene (Yr) Response		
			17	J	27
'WA'	2002	20%	R	R	R
'WA Yr17'	2006	12%	S	R	R
'Jackie'	2007	55%	R	S	R
'Jackie Yr27'	2008	<1%	R	S	S

The 'Jackie' pathotype dominated the population, despite only its second season of detection in Australia. This pathotype is adapted to triticales and became established early 2008 on long season triticales (carrying the *YrJ* resistance) and wheats sown for dual purpose grazing and grain. Since this pathotype dominated the *Pst* population, wheats carrying the *Yr17* resistance remained resistant, especially in the early phase of the epidemic. However the 'WA Yr17' pathotype re-emerged in spring and varieties carrying this gene were sprayed to ensure yield protection.

The first detection of the 'Jackie Yr27' pathotype in 2008 raises concerns for wheats carrying *Yr27*, ie Ruby, Merinda, Waagan. However the combined resistance of *Yr17* and *Yr27* in Livingston wheat will be expected to provide protection to all pathotypes detected in 2008.

Continuing studies to determine the resistances deployed in commercial agriculture, monitoring pathotype dynamics during seasonal development and clear communications with the extension community provides an important basis for disease control recommendations.

ACKNOWLEDGEMENTS

This work forms part of the Australian Cereal Rust Control Program funded by the Australian Grains Research and Development Corporation.

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Impact of sowing date on crown rot losses

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INTRODUCTION

Crown rot caused by the fungus *Fusarium pseudograminearum* (*Fp*) is a major constraint to winter cereal production in the northern cropping region especially under no-till farming systems (3). Yield loss from crown rot interacts heavily with moisture stress during grain-fill. One way to manipulate this interaction is through sowing time. Only two studies have ever examined this interaction under natural field infections which both found that earlier sowing increased the incidence of crown rot (1,2). An issue with these previous studies is that they are unable to differentiate seasonal interactions from the direct crown rot effects. An inoculated versus uninoculated experimental design, as suggested in (2), was adopted in this study to allow the direct effects of crown rot to be determined on yield and quality across three sowing dates.

MATERIALS AND METHODS

Three bread wheat varieties (EGA Gregory, Strzelecki and EGA Wylie) were used with the first two being longer season and rated as being more susceptible to crown rot and EGA Wylie a main season variety which has the best resistance rating. Plots of each variety were either uninoculated or inoculated with sterilised durum grain colonised by *Fp* at a rate of 2g/m of row. Plots of each treatment were then sown on three different dates at Tamworth in 2008 being: 1st sowing = 21st May, 2nd sowing = 10th June and 3rd sowing = 27th June. There were four replicates of each treatment which were blocked for sowing time with treatments randomised within each block. Hand samples were removed from each plot at physiological maturity to obtain pathology measures while yield and quality were obtained from samples collected using a small plot harvester.

RESULTS

Good rainfall occurred at Tamworth late in the season during grain-fill which prevented the formation of whiteheads in all treatments. There was no significant variety x inoculum or variety x sowing time x inoculum effect on yield given this good finish to the season. Sowing time had a significant impact on final grain yield in all three varieties with the average percentage yield reduction between the 1st and 2nd sowing for the three varieties being -9% and between the 1st and 3rd sowing -22.6%. Crown rot had less of an impact on yield at each sowing date causing -4.1% yield loss at 1st sowing, -3.4% 2nd sowing and -6.7% at 3rd sowing date.

Percentage screenings were also significantly affected by sowing time (1st to 2nd sowing date +1.0%; 1st to 3rd sowing date +3.5%). Crown rot also had a direct effect of increasing screenings at each sowing date with a trend towards increased negative impacts with delayed sowing (1st +0.7%, 2nd +1.5% and 3rd +1.7%).

There was no difference between the three varieties in the levels of infection initiated by *Fp* at any sowing date i.e. longer season varieties did not have greater numbers of plants infected irrespective of sowing time. In plots where no additional *Fp* inoculum was added (background infections) there was no difference in the percentage of plants infected at harvest between the three sowing times. When *Fp* inoculum was added, all sowing times resulted in around 80% of plants or greater being infected at harvest with the 2nd sowing time being

significantly higher at 94% than the other two sowing times. However, with both inoculum levels it was obvious that early sowing did not result in increased numbers of infected plants at harvest.

Although early or delayed sowing time did not impact on the percentage of plants ultimately infected by *Fp*, it did appear to influence disease expression as measured by the extent of basal browning (i.e. crown rot severity). Delaying sowing time significantly increased disease severity across the three sowing dates at both inoculum levels.

DISCUSSION

Sowing time and hence length of exposure to infection over the season did not result in different levels of plants being infected by *Fp* at harvest. The 2008 season was very conducive to infection with good soil moisture for much of the year. Certainly longer season varieties and earlier sowing did not increase susceptibility to infection.

Earlier sowing increased yield and reduced screenings irrespective of crown rot infection. The actual % yield loss to crown rot did not vary greatly between sowing times with each of the varieties. There was an indication that crown rot resulted in increased screenings with later sowings. The 2008 season was not overly conducive to yield and quality loss from crown rot but differences were still evident. It would be interesting to repeat this experiment in a season with a tougher finish. In theory, bringing grain-fill forward even 1–2 weeks may have a considerable impact on disease expression by limiting moisture and evaporative stress.

The major effect on yield and quality comes from the sowing time itself. Later sowing decreases yield potential and grain size and increases screenings. Adding crown rot into the picture on top of this further exacerbates these losses thus increasing the probability of downgrading. The % yield and quality losses attributable to crown rot were pretty consistent across the three sowing dates. If anything they got slightly worse with the later sowings. Hence, sowing earlier in the window, if soil moisture allows, maximises the genetic yield potential, grain size and limits screenings in a variety. This provides buffering from any detrimental effect that crown rot infection may then have.

ACKNOWLEDGEMENTS

Partial funding for this research was provided by the Grains Research and Development Corporation.

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Symptom development and pathogen spread in wheat genotypes with varying levels of crown rot resistance

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INTRODUCTION

Crown rot, caused by *Fusarium pseudograminearum* (*Fpg*), is an important soilborne disease of winter cereals. Complete resistance has yet to be reported in any wheat genotypes and hence is an ongoing issue for Australian wheat growers. In order to understand the nature of the partial resistance identified to crown rot we have examined the patterns of disease and pathogen spread in both susceptible and partially resistant tissues. Field trials were designed to study disease symptom development and localisation of *Fpg* hyphae in the bread wheat varieties Puseas, Vasco and Sunco, and the line 2–49.

MATERIALS AND METHODS

Inoculated field trials were conducted, using a randomised block design. Inoculum was placed in a band lying above the seed at sowing. Five plants from three replicates were harvested at approximately fortnightly intervals throughout the growing season. Leaf sheaths and internodes of the 1st 5 tillers were rated for disease using a scale from 0 to 4 as described in Wildermuth & McNamara (1), where 0 = no lesions evident and 4 = >75% of tissue lesioned. Following disease rating, each tissue piece from two replicates was surface sterilised and plated out on Czapek Dox agar. Plates were checked daily for 5 days after plating. Sites of colony emergence were marked with ink on the abaxial plate surface.

Disease rating data were analysed untransformed and the isolation counts were square-root transformed prior to analysis. Restricted Maximum Likelihood (REML) Variance Components Analysis was used to determine significance of the fixed factors harvest, genotype, tiller and the corresponding two and three way interactions. To determine where individual means were significantly different 95% confidence intervals of error were calculated for each analysed plant part.

RESULTS AND DISCUSSION

Differences in moisture conditions between the two field trials resulted in differences in overall plant development, extent of *Fpg* colonisation and symptom expression. The results of the second trial conducted under higher moisture conditions will be presented here.

Disease symptoms developed and *Fpg* was isolated from plant parts of all tillers of all genotypes. Statistically significant differences between genotypes were not expressed in the disease rating or isolation of *Fpg* from leaf sheath tissue in field trials even at the seedling stage (data not shown). Significant differences were seen between partially resistant and susceptible wheat genotypes in both disease rating and isolations from internode tissues and this could be detected soon after stem extension commenced (Figures 1 and 2).

Large differences in symptom expression were seen between genotypes in internode 1 around anthesis but not at maturity. This is an important observation as maturity is a favoured time

for rating field material for crown rot screening. At later harvests differences between genotypes were clearly expressed in higher internodes and at maturity lesions had developed as high as the 2nd internode in 2–49, 4th in Sunco, and the 5th internode in Puseas and Vasco. At maturity *Fpg* was consistently recovered from the 4th internode in 2–49 and the 5th in all other tested genotypes, indicating a delay in symptom expression in the infected 2–49 tissues.

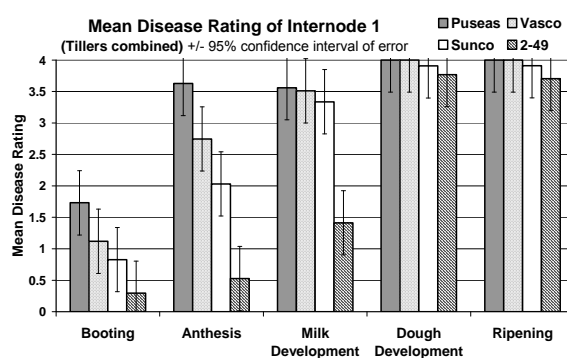


Figure 1. Mean disease rating of internode 1 at 13 (booting) to 22 weeks after planting (WAP) (ripening). n = 75 tillers

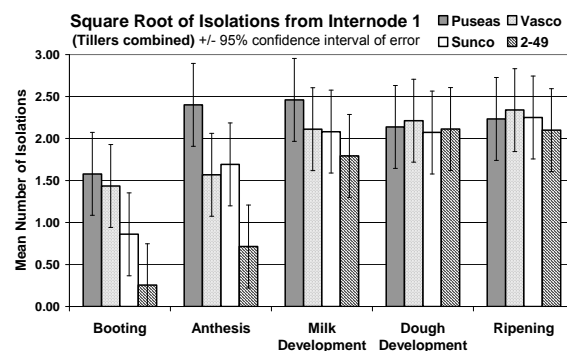


Figure 2. Mean isolations from internode 1 at 13 (booting) to 22 WAP (ripening). n = 50 tillers

CONCLUSIONS

Resistance was expressed as a slowing down of colonisation of plant parts in 2–49 and to a lesser extent in Sunco when compared to the susceptible genotype Puseas. Both colonisation and disease symptoms are initially slowed in young tissues of partially resistant genotypes however at later harvest times these same tissues may be as infected and symptomatic as the tissues of susceptible genotypes.

ACKNOWLEDGEMENTS

Financial support provided by GRDC PhD scholarship to CDP.

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Development of an eradication strategy for exotic grapevine pathogens

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INTRODUCTION

Eradication of exotic grapevine diseases can incur significant costs to growers and the industry using current strategies which include complete removal of affected and suspected vines. Alternative strategies need to be developed which optimise efficiency of the eradication process and minimise the economic cost of returning the crop to its previous quality and production levels (1). The endemic disease of grapevine, black spot (*Elsinoe ampelina*), was used as a model to develop a drastic pruning eradication strategy for the exotic disease black rot (*Guignardia bidwellii*). These pathogens have similar biology and epidemiology and as surface pathogens, inhabit fruit, leaves and shoots of grapevines producing similar looking lesions on these parts of the vine (2, 3).

MATERIALS AND METHODS

In 2006, a trial was established in the Sunraysia district of Victoria to develop and assess a drastic pruning protocol. Using a randomised block design, the trial comprised four table grape cultivars (Red Globe, Christmas Rose, Blush Seedless and Fantasy Seedless) as blocks. Plots consisted of three vines with standard two-bud spur pruning. Vines in each plot were either drastically pruned (as described below) or left as controls. Spacing between plots within rows was at least 7.3 m and between rows was 10.5 m.

Vines were inoculated in spring 2007 by spraying a suspension of *E. ampelina* conidia on new shoots with 2–4 unfolded leaves. Inoculations were conducted at three different times to cater for differences in phenology between the cultivars. Shoots were covered with polyethylene bags overnight to provide high humidity to promote spore germination and infection.

In July 2008, vines were drastically pruned as follows. Vines were cut at the crown using a chainsaw and excised material from above the crown was removed and placed in an excavated area about 25 m from the trial plots. The vineyard floor around the treated vines was raked and the debris was placed in the excavated area to be burnt and buried. Soil between vines was disc cultivated to bury any remaining debris. Trunks of the treated vines were drenched with lime sulphur using a back pack sprayer.

Canopy misters were used in the spring as new shoots emerged to provide conditions conducive for disease development. Vines were assessed for recurrence of symptoms in December 2008. Healthy sentinel vines in pots were placed strategically within and around the trial site during spring and early summer to detect any movement of the pathogen between plots or from external sources. After periods of 2–3 weeks, the potted vines were placed in a glasshouse, drip irrigated, incubated for 4 weeks at 22–28°C and inspected for symptoms.

A bioassay was conducted to determine if vine debris in the soil below vines was a source of inoculum for emerging shoots. Soil from the base of treated trunks was collected and organic debris was separated using a sieve. The debris was soaked in water overnight and the water was decanted and sprayed over

emerging leaves on potted grapevines (cv. Thompson Seedless). The leaves were incubated overnight as described above. The vines were assessed for symptoms 12 days later.

RESULTS

Assessment of the vines in December 2007 showed that 5–12 inoculated shoots on each vine had black spot leaf lesions and stem cankers. This indicated that the vines had sufficient infection to simulate an exotic pathogen incursion for eradication.

In December 2008, following the eradication, symptoms were recorded on all control vines and on 4 of 36 treated vines. On treated vines, each symptomatic shoot grew from the trunk within 20 cm of the ground. The bioassay indicated that symptoms were most likely caused by inoculum produced from vine debris remaining on the vineyard floor directly beneath low shoots.

Assessment of the sentinel vines revealed that there was no spread of disease between plots or from external sources.

DISCUSSION

As a result of the assessment following eradication, the protocol has been modified to include removal of lower shoots when regrowth occurs on vine trunks and the use of straw mulch on the vineyard floor. The revised protocol will be applied in the second year of the eradication trial in Australia and the assessment in December 2009 will determine if the eradication was successful. Validation of the protocol for eradicating black rot has been initiated in an infected vineyard in New York USA, where the disease is endemic.

This research has potential to save the Australian wine industry over \$18 million in lost production and vineyard re-establishment if there is an exotic disease incursion (R. Mcleod, unpublished data).

ACKNOWLEDGEMENTS

We would like to thank K. Clarke (DPI Vic), A. Loschiano and D. Sosnowski (SARDI) for technical assistance and the CRC for National Plant Biosecurity for funding this research.

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Green grassy shoot disease of sugarcane, a major disease in Nghe An Province, Vietnam

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INTRODUCTION

Sugarcane is a major crop in many South East Asian countries and provides an important cash crop as a rotation in a farming system often consisting of crops such as rice, corn, peanuts and watermelon. In contrast to first world countries, cropping areas in Vietnam are small with up to 24,000 farmers supplying sugarcane from 1ha plots to the local factory. This compares to around 250 farmers supplying each Australian sugar factory. In the mid-1990s, a new sugarcane disease called green grassy shoot disease (GGSD) was identified in Thailand. Characterised by the production of many small grassy tillers, and caused by a phytoplasma, the disease had severe consequences on crop yields. Several other diseases in neighbouring countries are also caused by phytoplasmas; these include white leaf disease (WLD) and grassy shoot disease (GSD). In 2006, symptoms of GGSD were identified in the NAT&L factory area, Quy Hop, Nghe An Province, Vietnam. This paper briefly describes GGSD symptoms and the current epidemic occurring in Vietnam.

GGSD

Symptoms. The disease is characterised by the production of many small green grassy tillers. These first appear at the base of mature sugarcane stools late in the cropping period; in this crop, yields are not unduly affected. Being a semi-perennial crop, second and third annual harvests (first and second ratoon crops) are made from the same planting. The following ratoon crops arising from an infested crop suffer very serious yield effects. Healthy ratoon shoots are replaced by profuse green, grassy shoots that lead to complete crop failure. Harvest yields often progress from 80 tonnes biomass per ha in a largely disease-free plant crop to 15 tonnes / ha in the first ratoon crop; second ratoon crops in susceptible cultivars often fail altogether. In contrast to GSD and WLD, there is no chlorosis in leaves of GGSD affected sugarcane.



Figure 1. Symptoms of GGSD in sugarcane crop (cultivar MY55-14) in Nghe An Province, Vietnam. Note the small green grassy tillers in the midst of normal ratoon shoots.

Causal agent. Research undertaken in Thailand suggests that a phytoplasma is the causal agent of GGSD.

Transmission. As a vegetatively propagated crop, infected planting material leads to diseased crops; the supply of disease-free seed-cane is essential for limiting disease spread. There are no recorded vectors for GGSD but circumstantial evidence, such as speed of spread, suggests a vector is likely to be associated with disease transmission.

Control. The most important control measures for GGSD are the termination of heavily diseased crops, the planting of new crops with disease-free planting material and the choice of the most resistant cultivars—though there are few resistant cultivars currently available in Vietnam. Further importation of germplasm into Vietnam is needed to select suitably-resistant cultivars. Research has shown that immersion of infested planting material in water maintained at 50C for 3 hours (HWT) leads to the elimination of the disease in >85% of the axillary buds. The selection of the cleanest planting material for HWT provides the best opportunity for producing disease-free nursery cane.

NAT&L sugar factory, Nghe An Province. The disease has been widely detected in the two most widely planted cultivars MY55-14 and ROC 10; ROC 10 is more susceptible than MY55-14. The disease quickly expanded beyond the initial finding with severe GGSD observed in >6,000ha of crops in early 2009; lighter infection has been widely observed across the sugar factory area. The sugar factory has pro-actively addressed the problem with incentives paid to farmers to eliminate badly diseased crops. Concurrently an intense extension program has been run by the factory in the local communes; over 175 commune meetings were staged from January to May 2009. In late April-early May 2009, there has been an expanded program, with further funding, focused on the elimination of infested crops in an attempt to further reduce disease spread.

DISCUSSION

The extent of the disease in the Quy Hop sugar factory area, the speed of spread and the effect on yield all suggest that GGSD is a very significant threat to sugarcane crop production in Vietnam. Not enough is known about the disease, including the nature of possible vectors, the resistance of cultivars to the disease, and potential replacement canes, and the distribution of the disease in Vietnam. There is a suspicion that GGSD also occurs in other Provinces of Vietnam, but at lower severity levels. Further research is needed, not only with GGSD but also to develop reliable diagnostic tools for GGSD, GSD and WLD. Findings of white leaves associated with diseased cane crops suggest that GSD and / or WLD may also be present in Vietnam. It is important that the status of the various pathogens is known to ensure appropriate control measures are applied.

ACKNOWLEDGEMENTS

We acknowledge the assistance provided by NAT&L factory staff in gathering information on this disease.

Molecular detection of *Mycosphaerella fijiensis* in the leaf trash of 'Cavendish' banana

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INTRODUCTION

Black Sigatoka (black leaf streak) caused by, *Mycosphaerella fijiensis* Morelet (anamorph *Paracercospora fijiensis* (Morelet) Deighton), the most destructive foliar pathogen of bananas globally. The disease is present in commercial plantations in Africa, Asia and Central and South America, where extensive fungicide applications are required for its control. The potential for *M. fijiensis* to be carried into countries free of the disease in leaf trash carried in commercial consignments is unknown. This study was undertaken to determine whether *M. fijiensis* could be detected in leaf trash in cartons of bananas imported from the Philippines to New Zealand.

MATERIALS AND METHODS

Samples of leaf tissue and banana skin were collected from cartons of a commercial consignment of bananas imported to New Zealand from the Philippines in December 2005. The samples were stored at -20°C until assayed in July 2006.

DNA extraction. DNA was extracted from 11 of the samples supplied (Table 1) using a QIAGEN DNeasy[®] Plant Mini Kit. Insufficient sample of S40 (particulate leaf material) was present for extraction. Samples of *M. fijiensis* (748 ex banana leaf, Tongatapu, Tonga) and *M. musicola* (yellow Sigatoka) (Mf589 [Cultures are held in the culture collection maintained by Dr R.A. Fullerton at Plant and Food Research, Mt Albert Research Centre, Auckland.] ex banana leaf South Johnston, Queensland) were used as control samples and had DNA extracted from mycelium growing on a potato dextrose agar plate. DNA was quantified using a NanoDrop spectrophotometer. DNA extracts were kept at -20°C.

Table 1. List of banana leaf, floral and trash samples from which DNA was extracted. DNA was also extracted from *Mycosphaerella fijiensis* and *M. musicola* cultures.

Sample	Type
S2	Floral
S7	Edge of leaf?
S31	Leaf material
S32	Leaf material
S36	Particulate trash?
S39	Leaf material
S56	Stem/petiole on fruit
S113	Fruit spots under trash
S155	Particulate trash
S348	Unknown
S351	Unknown
Mf 748	<i>Mycosphaerella fijiensis</i> culture Fullerton
Mf 589	<i>Mycosphaerella musicola</i> culture Fullerton
Banana leaf	Healthy glasshouse grown plant in NZ

PCR protocol. Samples were initially amplified using primers MF137 and R635 (1). These primers were found to be not specific for *M. fijiensis* and alternative primers were sought, MFFor and R635-mod (Henderson et al. unpublished). These primers are designed to amplify part of the internal transcribed spacer (ITS) regions between the 18S and the 28S rDNA subunits of *M. fijiensis*. Prior to amplifying the extracted DNA, the minimum detection limit of *M. fijiensis* for the PCR protocol being used was determined using DNA extracted from a culture

of known identity (Mf 748). This was shown to be 0.243 fg/μL (1 fg = 10⁻¹⁵ g) of pure *M. fijiensis* DNA. When assaying the extractions from leaf trash, a PCR product of approximately 1050 bp indicated a positive amplification of *M. fijiensis* (Figure 1). Where a positive result was achieved, the PCR reaction was repeated a minimum of three times. A control of healthy, uninfected banana leaf was also included in reactions.

Sequencing. Direct sequencing was carried out to confirm the identity of the amplified products. PCR products were purified using a QIAGEN MinElute PCR Purification Kit. The PCR product was fluorescently labelled using a BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA). Each 10 μL reaction contained approximately 25 ng/μL of PCR product, 2 μM primer (ITS-1 or ITS-4), 2 μL terminator-ready reaction mix and the volume made up with sterile Milli-Q water. Sequences obtained were compared with those in the database GenBank[®] using the Basic Local Alignment Search Tool (BLAST), BLASTN.

RESULTS AND DISCUSSION

Four of the 11 tissue samples consistently yielded a PCR product using *M. fijiensis* specific primers MFFor and R635-mod (Figure 1). They were: S2 (floral), S31 (leaf material), S36 (particulate trash) and S56 (stem or petiole). Sequenced products were homologous (at least 99%) with *M. fijiensis* sequences lodged in GenBank. This study has shown that *M. fijiensis* was present in fragments of leaf trash found in cartons of banana fruit imported into New Zealand from the Philippines. The viability of the organism within the sample cannot be ascertained from these tests nor can the quantity of *M. fijiensis* be verified using these techniques.

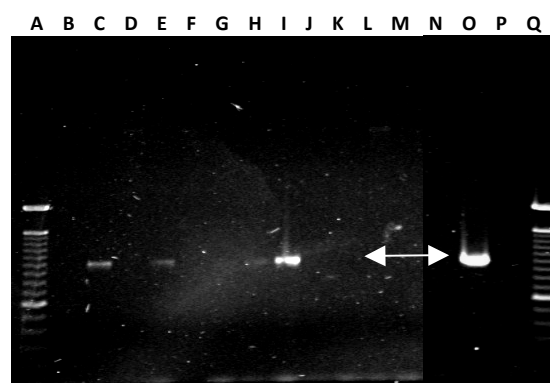


Figure 1. Amplified products of *Mycosphaerella fijiensis* using primers MFFor and R635-mod. Reaction used 5 μL DNA per reaction. Lane A:100 bp ladder; B:Banana leaf; C:S2; D:S7; E:S31; F:S32; G:S39; H:S36; I:S56; J:S113; K:S155; L:S348; M:S351; N:Mf589 yellow sigatoka; O:Mf748 black sigatoka; P:blank (negative control); Q:100 bp ladder. Double-ended arrow indicates product of approximately 1050 bp. Deteriorating DNA lessened the band brightness for some samples. Note: gel has been cut.

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Optimising responses to incursions of exotic plant pathogens

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INTRODUCTION

Biological, spatial and economic data, linked through modelling, can assist in optimising responses to incursions of exotic plant pathogens. The approach allows predictions of the behaviour of linked biological and agronomic systems within defined bounds despite many uncertainties involved in individual parameters. Uncertainties are to be expected because each incursion of an exotic pest into a new environment is a novel situation for which there may be no precedents. The biological and agronomic parameters having the greatest impact can be identified, and the response designed to optimise the benefit:cost ratio.

The value of this approach is shown in examples of two relatively recent incursions into Australia by exotic pathogenic nematodes; (a) *Bursaphelenchus hunanensis*, a relative of the Pine Wilt Nematode (1), (b) Potato Cyst Nematode (PCN) (2).

MATERIALS AND METHODS

The model initially simulated possible scenarios for the arrival, establishment, and expansion of the geographic range of a pest in the absence of biosecurity measures. The effects of various measures were then added and the results compared with the first run.

The model was a stochastic simulation model using random number generators to simulate chance or random events. Probability distributions were used as parameters within an abstract model rather than point estimates, and a Monte Carlo algorithm used to sample from each of these distributions (3).

Many parameters were used to estimate the ecological processes of establishment, spread, population growth and crop damage, together with their economic consequences in terms of crop yields, testing for disease, and control measures. Each parameter was given one of a number of statistical distributions with a defined mean or modal value, depending on the distribution chosen. In each of the 5,000 iterations of the model, one value was randomly sampled across the range of each distribution. The model used Markov chains to estimate transitional probabilities between time periods of 1 year. The model was run over 20 years and used a standard discount rate of 8% (a margin of 3% on top of a real risk free rate of 5%).

RESULTS

Impacts of both pests studied were large over the time period considered. Under most possible scenarios, annual impact rises steeply initially, followed by slower growth, before eventually declining (Fig. 1). Raw crop losses in the field were only a small proportion of the aggregate impact. Parameters had different effects and time courses on the aggregate impact of the pests. Potential rate of geographic expansion of the pest was important, but the cost of testing for the pest during its expansion was also important. This cost occurs soon after invasion; it can be largely independent of the actual expansion rate or range, but is affected by the accuracy and efficiency of the test. Efficacy of testing affects the impact of a pest on other crops occurring in the region. Cost of mitigation of the pest may be large, and the failure rate of control is an important cost.

Impacts related to trade in the crop, both in terms of quantity and value are highly uncertain, but under most scenarios are

highly significant. With increasing distances from production to market, the chances of barriers to trade arising or loss of markets following arrival of a pest are increased. Disinfestation and certification costs were substantial in the long term.

DISCUSSION

Rapid initial rise in impact of pests makes early detection and action desirable, even when there is great uncertainty over the future behaviour of the pest. The substantial impact beyond lost crop production means that eradication or other control measures are often the best option. The problem is that cost of this strategy precedes any benefits. Benefits of control programs may be wider than the direct crop losses, so wider contributions to costs may be justified.

The predicted decline in annual impacts may be largely related to discount rates and this requires further investigation since the real costs of many forms of pest control, eg chemicals, are increasing, along with environmental, social and regulatory costs.

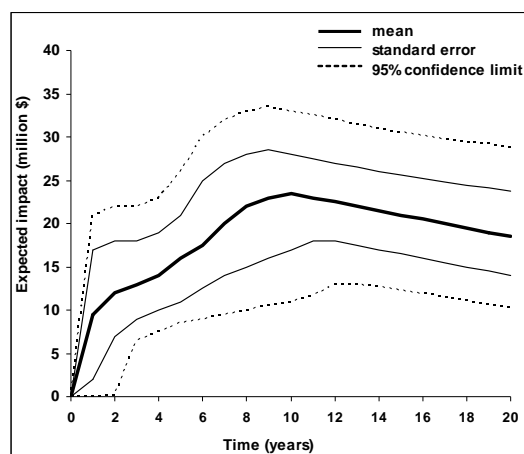


Figure 1. Simulated impact of PCN in Australia.

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The influence of soil biotic factors on the ecology of *Trichoderma* biological control agents

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INTRODUCTION

The influence of environmental factors such as pH, moisture, temperature and other abiotic factors such as fertiliser and pesticide application on the establishment, proliferation and persistence of biocontrol agents in the field has been intensively investigated (1). However, there is little understanding of the nature of biotic influences which are likely to play an equally important role in determining the nature of the biocontrol outcome. This paper reports on a preliminary study that examines the effect of common soil microbes on two biocontrol agents, *Trichoderma atroviride* LU132 active against onion white rot and *Trichoderma hamatum* LU593 active against Sclerotinia lettuce rot (2).

MATERIALS AND METHODS

Soil microbes. Forty-eight microbes representing 11 fungal genera (*Acremonium*, *Alternaria*, *Aspergillus*, *Beauveria*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Metarhizium*, *Paecilomyces*, *Penicillium*, *Verticillium*), seven bacterial genera (*Agrobacterium*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Flavobacterium*, *Paenibacillus*) and four actinomycete genera (*Actinomyces*, *Arthrobacter*, *Rhodococcus*, *Streptomyces*) were obtained from NZ culture collections (Lincoln University, Landcare Research, AgResearch).

Dual culture assays. Test microbes were inoculated 3d prior to or simultaneously with the *Trichoderma* on 9 or 15cm diameter PDA plates. An inoculum plug of the test microbe was placed 3cm apart from the *Trichoderma* in the centre of the plate. Colony interactions were monitored every 24h until *Trichoderma* colony growth stopped or was constrained by the edge of the plate. *Trichoderma* colony area (mm²) was measured and percentage inhibition compared to the *Trichoderma* control calculated.

Soil pot assays. Inocula of six test microbes were produced on rice grains and incorporated into Templeton silt loam soil in pots to give 10⁶ cfu/g soil. *Trichoderma* was applied to the soil (10⁶ cfu/g soil) as a granular formulation (Agrimm Technologies Ltd). Pots were incubated at constant temperature and moisture for 30d. At weekly intervals, soil samples were taken from three random spots in each pot and *Trichoderma* population counts (cfu/g soil) determined using soil dilution plating on *Trichoderma* selective medium.

Statistical analyses. Data was analysed using one-way ANOVA and treatment means compared using Fishers LSD.

RESULTS

Dual culture assays. Co-culture on PDA revealed six fungi and one bacterium that significantly inhibited *Trichoderma* colony growth (Table 1). Greatest inhibition (>85%) occurred with *Aspergillus niger* and *Paecilomyces lilacinus* for *T. atroviride* and *T. hamatum*, respectively. In general, *T. hamatum* was less sensitive than *T. atroviride* to the test microbes, in particular to *C. globosum*.

Soil pot assays. *T. atroviride* populations were significantly reduced in soil treated with *Alternaria*, *Aspergillus*, *Metarhizium*,

Paecilomyces and *Daldinia* (Fig. 1). *T. hamatum* was less sensitive to the test microbes but the trend was similar (data not shown).

Table 1. Percentage inhibition of *Trichoderma* colony growth after 10d dual culture with test microbes

Test microbes	<i>T. atroviride</i>	<i>T. hamatum</i>
<i>Alt. alternata</i>	94.7 a*	72.8 c
<i>P. lilacinus</i>	84.6 b	85.1 a
<i>Asp. niger</i>	96.5 a	82.2 ab
<i>C. globosum</i>	87.2 b	44.5 d
<i>M. anisopliae</i>	82.5 b	74.2 bc
<i>D. eschscholzii</i>	56.4 c	51.2 d
<i>Agrobacterium</i> sp	67.1 c	31.3 e

* Values within columns followed by the same letter are not significantly different.

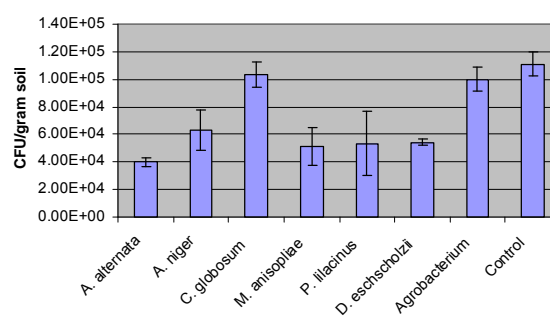


Figure 1. *T. atroviride* population (cfu/g soil) after 30d in soil treated with different test microbes.

DISCUSSION

Six fungi and one bacterium significantly inhibited *Trichoderma* colony growth on agar plates with differential sensitivity observed between the two *Trichoderma* strains. Preliminary studies suggest the inhibition is due to the production of antifungal metabolites by the test microbes. The high inhibition observed in culture was not reproduced in the soil assay where five of the seven test microbes reduced *Trichoderma* populations but only by ten-fold. Since test microbe populations in the field are likely to be lower than those used here, the results likely overestimate the potential negative impact on *Trichoderma* biocontrol agents applied to soil. However, further work examining the effect of the test microbes in different soil types is needed since metabolite production by the test microbes may be influenced by soil abiotic factors.

ACKNOWLEDGEMENTS

Funding for this project was provided by the NZ Tertiary Education Commission.

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Understanding *Trichoderma* bio-inoculants in the root system of *Pinus radiata*

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INTRODUCTION

The genus *Trichoderma* are beneficial soil-borne fungi and a well known source of biological control agent active against a wide range of crop diseases, including those of pine trees (1). Several isolates of *Trichoderma* have been shown to improve establishment and reduce pathogen infection of *Pinus radiata* in the nursery and in forestry plantations (2). Three isolates of different *Trichoderma* species were selected for this study. *T. hamatum* (LU592) and *T. harzianum* (LU686), known to stimulate growth and improve establishment of *P. radiata* seedlings, and *T. atroviride* (LU132) which had no stimulatory activity. To enable more predictable and effective use of *Trichoderma* bio-inoculants, their establishment and population dynamics was determined. In addition, the effect of each isolate on *P. radiata* seedling vitality and growth was assessed.

MATERIALS AND METHODS

Each *Trichoderma* isolate was applied either as a seed coat formulation (4×10^5 spores/seed; SC) or a spore-suspension (5×10^6 spores/pot; SA) sprayed directly after sowing the *P. radiata* seeds. *P. radiata* seeds were grown in root-pruning containers for 7 months under conditions reflecting those used in the commercial PF Olsen nursery. Health and growth assessments included mortality rate, shoot height and shoot and root dry weight measurements. During the 7 month trial period, *Trichoderma* populations were enumerated in the bulk potting mix, rhizosphere, rhizoplane and endorhizosphere subsystems by dilution plating. At the 20 week assessment, recovered *Trichoderma* colonies were identified using morphological and molecular techniques to differentiate between introduced and indigenous species. A large-scale experiment was set up at the PF Olsen nursery under commercial conditions to verify the results for LU592.

RESULTS

T. hamatum LU592 performed the best out of the three introduced isolates. Seedling mortality rate was reduced from 5.2% for the control to 0.2% for LU592 and 0.4% for *T. harzianum* LU686 SC. LU592 and LU686 SC also increased shoot height by 17% and 11%, respectively. Results also indicated that *T. atroviride* LU132 increased the root/shoot ratio.

Trichoderma populations of all SA treatments were significantly higher in the rhizosphere (by 2.1 to 3.3 times) compared with the control. Applied *Trichoderma* spp. could be differentiated from indigenous isolates by colony morphology and confirmed by molecular sequencing. Introduced *Trichoderma* isolates could be detected even though overall *Trichoderma* populations did not reveal significant differences to the control. In the rhizosphere, introduced isolates established with levels of ~20% for LU132 SA and LU592 SC. *T. harzianum* LU686 was not recovered from the rhizosphere after 20 weeks. *T. hamatum* LU592, when spray applied, was the only isolate clearly dominating all four subsystems bulk potting mix, rhizosphere, rhizoplane and endorhizosphere.

T. hamatum LU592 as a seed coat and spray application significantly increased shoot height, shoot and root dry weight and stem diameter compared with the control in the large-scale experiment (Table 1).

Table 1. Increase (%) in *P. radiata* seedling growth parameters by *T. hamatum* LU592 compared with the control. All values significant at $P > 0.05$ from the control.

Growth factor	Seed coat	Spray Suspension
Shoot height	7.4	9.5
Dry weights - roots	17	21
- shoots	23	24
Stem diameter	9.0	9.4
number root tips	11	n.s.

n.s. = not significant

DISCUSSION

Both *T. hamatum* LU592 and *T. harzianum* LU686 increased the growth of *P. radiata* seedlings, with the subsequent large-scale experiment confirming the growth promotion effects of LU592. The spray application performed slightly better than the seed coat application. This reduction in seedling mortality and increase in seedling growth represents a substantial economic benefit to the industry.

The spray application method clearly promoted the establishment of the introduced isolates in the root system of *P. radiata*. *T. harzianum* LU686 was found to be an early rhizosphere coloniser (declining after 12 weeks). Strong rhizosphere competence was identified for *T. hamatum* LU592. The ability of *Trichoderma*, LU592 in particular, to establish in the rhizosphere and penetrate the roots is a crucial indicator of beneficial activity (1).

LU592, being the most effective isolate at colonising all *P. radiata* root subsystems, was selected for more detailed ecological studies using a genetically marked strain. Future experiments will focus on the use of a fluorescently marked isolate of LU592 to verify rhizosphere competence, examine spatio-temporal distribution within the rhizosphere and determine endophytic activity including interactions with ectomycorrhizae.

ACKNOWLEDGEMENTS

This study is part of the Ecosystem Bioprotection program LINX0304 funded by the NZ Foundation for Research Science and Technology (FRST). We would like to thank PF Olsen for nursery access.

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A bioassay to screen *Trichoderma* isolates for their ability to promote root growth in willow

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INTRODUCTION

Trichoderma species have been shown to increase the biomass of both root and shoots of a range of plants including willow (1). These positive benefits of biocontrol agents have been attributed to antibiotic production, parasitism or competition of pathogenic fungi, activation of the host defence response, and possibly the direct stimulation of plant growth.

Cuttings of some plant species such as *Pinus radiata* are slow and difficult to root resulting in poor plant establishment. The Bio-Protection Research Centre culture collection has a large number of *Trichoderma* isolates and a bioassay to rapidly screen a selection of these isolates for their ability to promote root growth and establishment of cuttings was investigated. Willow was chosen as a model system because of its ease and speed to produce roots, enabling rapid screening of potential isolates. This paper describes the development of this assay.

MATERIALS AND METHODS

Dormant cuttings of *Salix x matsudana* willow, approximately 300 mm in length, were collected in July and August. Cuttings were given a two hour soak in tap water prior to storage in plastic bags at 4°C until required for treatment and planting. Representative isolates (65 in total) from a range of *Trichoderma* species from the Biocontrol Microbial Culture Collection (Bio-Protection Research Centre, Lincoln) were grown on PDA (pH 4.0) to produce inoculum (conidia). Conidia were harvested in reverse osmosis water, filtered through Mira cloth (22–25µm) and added to 0.5% methyl cellulose to produce an inoculum concentration of $1 \times 10^6 \text{ mL}^{-1}$.

The trial was separated into four experiments set up on different days. Each experiment included three control/standard treatments (methyl cellulose (0.5%), fulvic acid (0.3%) and Thiram (12 g L⁻¹)). Willow cuttings were dipped in each treatment (*Trichoderma* spore suspensions, controls) for 10 minutes, except Thiram (4 minutes as per manufacturer's recommendation). Treated cuttings were planted into individual planter bags (special Long PB ¾, 64 x 64 x 300 mm) filled with a pine bark, sand, pumice mix (50, 30, 20% respectively). The trial was laid out in a stratified random block design consisting of 4 blocks and 40 replicates per treatment. Plants were assessed for root development 35 days post planting with the following measurements recorded; cutting girth, cutting length, number of root initials and number of shoots. Statistical analysis was by ANOVA and included a range of parameters including root dry weight, root dry weight per unit volume cutting and root dry weight per root initial.

RESULTS/DISCUSSION

This bioassay proved successful for screening a large number of *Trichoderma* isolates for their ability to promote root growth on willow. In general, the majority of isolates had no effect on root promotion compared to the methyl cellulose control. A small number of isolates reduced root growth. However, three of the 65 *Trichoderma* isolates screened significantly promoted root growth (up to 40% more total root dry weight) compared to the methyl cellulose control. These isolates performed well when other parameters were examined with increased root:shoot

ratio and root weight per root initial. Figure 1 presents example data from experiment one demonstrating the ability of *Trichoderma* isolate T6 to increase all measured parameters.

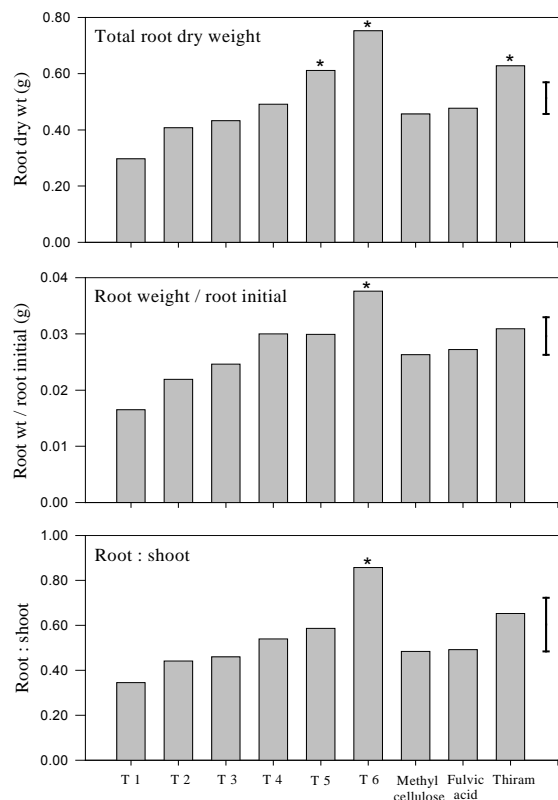


Figure 1. The effects of six *Trichoderma* isolates (T1-T6) on root weight/root initial, root/shoot ratio, and total root dry weight in willow compared to the control treatments methyl cellulose, fulvic acid, and Thiram for experiment 1. 5% LSD bars are shown (* indicates treatments significantly better than the methyl cellulose control).

These three isolates plus others which showed potential for promoting root growth will be further evaluated in additional experiments on other plant host cutting/seedling systems.

ACKNOWLEDGEMENTS

We wish to thank Candice Barclay, Bronwyn Braithwaite, Prashant Kumar Chohan, Emily Duerr, Stuart Larsen, Kirstin McLean, Mana Ohkura, for their technical assistance.

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Biofumigation for reducing *Cylindrocarpon* spp. in New Zealand vineyard and nursery soils

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INTRODUCTION

The soil-borne disease, *Cylindrocarpon* black foot disease (BF), is reported as the cause of decline of young grapevines in new and replanted vineyards world-wide. *Cylindrocarpon destructans*, *C. liriodendri* and *C. macrodidymum* have been found to be equally associated with this disease in New Zealand vineyards and nurseries.

Brassica species contain significant quantities of the thioglucoside compounds known as glucosinolates (GSLs). When GSLs are hydrolysed by the myrosinase enzyme present in *Brassica* tissue, volatile isothiocyanates (ITCs) are produced [1]. ITCs are known to have broad biocidal activity in the suppression of pathogenic fungal species, nematodes, weeds, and certain insect species [2]. The principal suppressive effect of brassica amendments on soil-borne diseases occurs at flowering, immediately after their maceration and incorporation into soil [3]. ITCs are also exuded from the roots of brassicas throughout their growth. The efficacy of *Brassica* spp. for control of *Cylindrocarpon* spp. in grapevines was tested in field experiments.

MATERIALS AND METHODS

A preliminary experiment used crops of mustard (*Brassica juncea*), rape (*B. napus*) and oats (*Avena sativa*). They were grown for 5 weeks in an infested site, where young vines previously grown had been infected with the above *Cylindrocarpon* spp. The brassica crops were cultivated into the soil and the area covered with polythene. After 2 weeks, callused rootstock cuttings (varieties 101–14 and 5C) were planted in the treated soil in a randomised split plot design (5 plots per treatment) according to standard nursery practices. The plants were grown for 9 months, harvested and infection assessed by isolation onto potato dextrose agar. *Cylindrocarpon* spp. were identified morphologically after 7–10 days incubation at 20°C.

The second experiment used 3 mustard treatments (Trt 1–3)

- Trt 1. Mustard meal cultivated into the soil.
- Trt 2. Grown once to flowering with cultivation.
- Trt 3. Grown twice to flowering with cultivation each time.

When Trt 3 was flowering (4 weeks), the entire field was inoculated with *Cylindrocarpon* spp. grown on wheat grains (3 isolates of each species) and 2 days later the mustard plants were cultivated into the soil and the area covered with polythene. After 3 days the polythene was removed and mustard seed was sown for Trt 2 and Trt 3 and grown to flowering. At this time, the mustard meal (Trt 1) was broadcast and all 3 treatments were incorporated into the soil. The area was covered with polythene for 2 days then callused cuttings of rootstocks 101–14 and 5C (20 per plot) were planted in a randomised split plot design (5 plots per treatment). The plants were grown for 10 months then infection assessed as above. Analysis of data was with a general linear model appropriate to the split-plot randomised block design.

RESULTS

In the preliminary experiment (Fig 1a), although not significant ($P=0.137$), disease incidence (DI) was reduced by the mustard treatment in rootstocks 101–14 and 5C (11 and 43%,

respectively). DI was decreased in rootstock 5C by the oats treatment but increased in 101–14 and increased in both rootstocks by the rape treatment. The mustard treatment was further tested in a second experiment (Fig 1b). Again, treatment effects were not significant for DI ($P=0.359$), which was reduced by Trt 1 and 3 in both rootstocks and in 5C by Trt 2. Overall, DI was reduced by the mustard treatments in rootstock 5C by more than 41% and in experiment two DI was reduced by Trt 1 and Trt 3 in rootstock 101–14 by 30 and 18%, respectively.

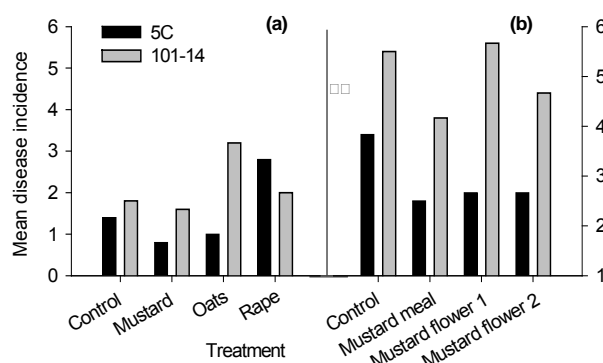


Figure 1. Mean *Cylindrocarpon* incidence in grapevine varieties 101–14 and 5C after cultivation of (a) brassica and oat crops and (b) mustard as crops and mustard meal.

DISCUSSION

The results indicate that biofumigation with mustard was effective for reducing infection by *Cylindrocarpon* spp. particularly in rootstock 5C, but less so in 101–14, which is more susceptible to BF disease. Overall, treatments 1 and 3 were able to reduce disease incidence by 36 and 27%, respectively. Despite the lack of statistical significance (which may be due to high variability), these findings suggest that mustard treatments may be a highly effective method for the control of BF disease.

Biofumigation may well be a natural, effective, and economical way to eliminate pests and diseases in vineyard and nursery soils, to improve soil structure and soil organic matter, and to increase soil microbial activity. A key to improving the efficacy of biofumigation in the field lies in the development of application technologies that macerate and incorporate the biofumigant evenly in soils, in addition to incorporating it under optimal edaphic conditions for release of ITCs [3].

ACKNOWLEDGEMENTS

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Crown rot of winter cereals: integrating molecular studies and germplasm improvement

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INTRODUCTION

Crown rot of winter cereals is a major constraint on grain production across most growing regions in Australia, particularly where stubble retention is practiced to maintain soil structure and retain soil water. The predominant cause of this disease is infection with *Fusarium pseudograminearum* (*Fpg*), although in some southern areas *Fusarium culmorum* infections are also significant. These *Fusarium* species are able to grow saprophytically on stubble remnants over the summer and provide inoculum for crop infection in the following season. Losses due to crown rot are highest in seasons featuring a dry finish in which maturing plants experience water stress, with symptoms including basal stem browning and white heads bearing no grain.

Control of this disease is challenging and is currently based on management practices centred on crop rotation strategies. At present, there are no resistant commercial varieties of bread wheat, durum or barley available for deployment. Durum wheats are particularly susceptible.

We are currently undertaking a long-term collaborative research program which aims to:

- characterise known resistance sources
- develop molecular markers for quantitative trait loci (QTL) to assist selection in breeding programs
- transfer QTL for resistance from hexaploid (bread) to tetraploid (durum) wheats
- pyramid resistance in bread wheats
- understand the fundamental biology of this host/pathogen interaction.

Central to the task is an integration of laboratory and field-based investigations to ensure outcomes that not only advance our knowledge but also reduce yield losses and increase management options for primary producers.

Here we report on recent successes in pyramiding sources of partial resistance and discuss progress in transferring resistance from hexaploid sources into a durum background.

METHODOLOGY

Two doubled haploid wheat populations produced from crosses of partially resistant parents, Sunco/2-49 and 2-49/W21MMT70 were evaluated for resistance to crown rot using a standard seedling pot test inoculated with a mixture of aggressive *Fpg* isolates(1). Based on genetic maps constructed from SSR and DArT markers, QTL for resistance were then identified.

Crosses between hexaploid wheat lines with partial resistance and a range of durum lines were obtained from Dr Ray Hare, NSW DPI. These materials were field grown near Tamworth NSW in *Fpg* infected plots through to the F7 generation and assessed for crown rot susceptibility each season.

RESULTS AND DISCUSSION

To date, a wide selection of resistance sources have been partially characterised and quantitative trait loci (QTL) identified (2, 3, 4). Inoculated seedling and field trials indicate overlapping sets of loci that contribute at these different stages of development. These partial sources of resistance contain largely different sets of QTL which suggest that improved resistance may be obtained by gene pyramiding. Results from QTL analysis of the Sunco/2-49 and 2-49/W21MMT70 populations following seedling trials indicate that the more resistant lines inherited the major QTL from each parent and that a number of lines in the 2-49/W21MMT70 population expressed significantly higher resistance than either of the parents. Hence pyramiding of independent resistance sources can produce significant improvements in the resistance of derived progeny towards crown rot.

Marker analysis of hexaploid x tetraploid crosses shows that the bread wheat markers were readily transferred to the progeny. Furthermore even after several generations these markers remained linked to the resistance character and were independent of remnant D genome material. Crosses of durum wheats with the hexaploid resistance sources significantly reduced the disease severity in derived materials, demonstrating the potential of this approach for improving the resistance of durums to crown rot.

ACKNOWLEDGEMENTS

We acknowledge the contributions of Dr Graham Wildermuth and Dr Ray Hare to this work. This work was funded by the Grains Research and Development Corporation (GRDC).

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Infection of wheat tissues by *Fusarium pseudograminearum*

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INTRODUCTION

Crown rot of wheat, caused by *Fusarium pseudograminearum* (*Fp*), is a serious disease threat across the Australian wheat belt. Currently control of this disease relies on farming practices (e.g. crop rotation) and planting of less susceptible cultivars. 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, with the majority of current screening methods being based on seedling scoring. 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).

The current project aims to assess growth of *Fp* during crown rot development across partially resistant and susceptible wheat lines in order to determine key elements in the progress of disease and when resistance mechanisms are induced. Current disease rating systems for seedlings rely heavily on browning of leaf sheaths and tiller bases (2). Our current investigations are centred on the relationship between the expression of these visible disease symptoms and the extent of fungal infection. These studies are also comparing the progress of fungal spread in both susceptible and partially resistant wheats. The increase in fungal load in each inoculated host genotype has been measured using a quantitative real time multiplex polymerase chain reaction (PCR) assay, allowing simultaneous detection of both pathogen and host DNA. Microscopy of infected wheat tissues is also in progress.

MATERIALS AND METHODS

Inoculation. Two week old seedlings were inoculated using a 10^6 conidia per mL suspension (3). Seedling tissues (particularly leaf sheaths) were harvested at different time points after infection, with four host genotypes being compared (Table 1).

Table 1. Genotypes tested and their resistance rating from field trials.

Genotypes	Crown Rot Resistance Rating
2-49	Partially Resistant
Wylie	Modestly Susceptible
Gregory	Susceptible
Pumas	Highly Susceptible

DNA Extraction and Multiplex Quantitative PCR. DNA was extracted using the DNeasy Minikit (Qiagen). Primers and probes were designed from Genbank sequences with Primer3 software using the translation elongation factor (TEF)- α sequence of *Fp* and the TEF-G sequence of wheat. PCR results were normalised by expressing *Fp* DNA content relative to the host DNA.

Microscopy. Fixation and clearing of tissues was performed as described in (4). Differential staining used safranin and solophenyl flavine dyes. Viewing of tissue was performed using a fluorescence microscope (Nikon Eclipse) under the UV-2A filter.

RESULTS AND DISCUSSION

A strong relationship was observed between visual rating scores of wheat leaf sheaths and the normalised *Fp* DNA (Fig. 1). This demonstrates that the degree of visual discolouration of wheat leaf sheaths correlates with the quantity of *Fp* mycelium present in the tissue, validating visual rating systems of basal discolouration (2,3) as a relative estimation of tissue infection levels across a seedling trial.

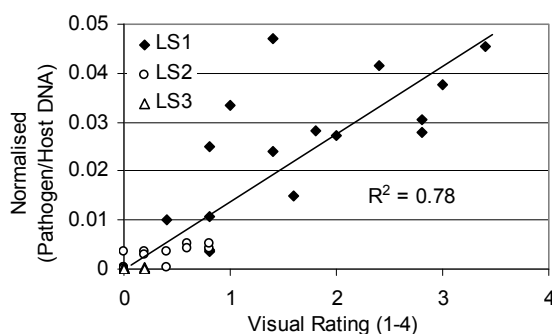


Figure 1. Comparison of levels of normalised *Fp* DNA and visual rating scores of leaf sheaths (LS) 1, 2 and 3 of the four host genotypes at 7 days after inoculation.

Observation of *Fp* growth at increasing periods after inoculation has revealed significant differences in growth of *Fp* in seedlings of the four standard genotypes.

Microscopic assessment of *Fp* growth has observed intra- and inter-cellular growth associated with the leaf sheath epidermis, including trichomes and stomata. Current investigations are examining growth of mycelium in vascular tissues of expanded tillers.

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Monitoring sensitivity to strobilurin fungicides in *Blumeria graminis* on wheat and barley in Canterbury, New Zealand

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INTRODUCTION

Strobilurin fungicides (Quinone 'outside' Inhibitors (QoI)) act at the Qo binding site of the cytochrome bc1 complex in target fungi (1). Because QoIs have a specific, single-site mode of action, there is a greater risk of developing resistance to these types of fungicides than to multi-site inhibitor fungicides. QoIs were first commercialised in 1996 and have since been widely used overseas and in New Zealand to control various plant diseases. QoI-resistance occurred in Europe in 1998. QoI resistance has been encountered in 29 different pathogens to date (2). In most cases, QoI resistance is conferred by a single point mutation in the cytochrome b gene at the amino acid codon 143, causing glycine to be replaced with alanine (G143A).

Sensitivity to QoIs in the pathogens causing powdery mildew of wheat (*Blumeria graminis* f. sp. *tritici*) and barley (*B. graminis* f. sp. *hordei*) was investigated in Canterbury, New Zealand cereal crops during two growing seasons (2004–05 and 2005–06).

MATERIALS AND METHODS

Three different surveys (spring 2004, autumn 2005 and spring 2005) were carried out using two methods of spore collection. In the first method, isolates of powdery mildew were collected from infected plants, bulked up in a glasshouse and tested on detached leaves of barley and wheat either treated with kresoxim-methyl or left untreated. Numbers of colonies formed on the leaves were counted. In the second method young trap seedlings of wheat (cv. Kotare) or barley (cv. Cask), either treated with kresoxim-methyl or left untreated, were exposed to the air spora at a specific location for 5–7 days, then placed in a glasshouse until lesions appeared. At each site, 15 untreated and 15 treated pots, with 10 plants per pot, were used. Lesions on untreated and treated leaves were counted, and colonies that grew on treated leaves were kept in isolation chambers for further analysis on detached leaves. There were a total of 33 wheat and 28 barley sites over the two seasons. Data (only spring 2005 results shown) were analysed using a log-linear model for count data with GenStat.

Fifteen isolates of *B. graminis* f.sp. *tritici* collected from wheat plants at six sites and 12 isolates of *B. graminis* f.sp. *hordei* collected from barley plants at six sites in the three surveys were tested for presence of mutation G143A. These isolates had shown increased resistance to QoIs in the lab tests. DNA was extracted from the lesions and the cytochrome b -gene was amplified with PCR. For qualitative analysis the product was digested with restriction enzyme *Sat1* to detect the presence of the gene G143A for resistance and for quantitative analysis the PCR-product was analysed using pyrosequencing.

RESULTS

Spring 2004. Trap plant studies indicated that in nine out of ten sampled locations, wheat powdery mildew colonies were able to grow on fungicide-treated plants. The results for barley powdery mildew showed that in seven out of nine locations, barley powdery mildew colonies were able to grow on fungicide-treated plants. No resistance was detected in any of the isolates collected from infected plants in the field.

Autumn 2005. Survey results indicated no resistance to wheat powdery mildew, but resistant barley powdery mildew isolates were found at all five sites where trap plants were placed, and four out of six sites where isolates were collected from infected plants in the field.

Spring 2005. Trap plant results indicated that resistant wheat powdery mildew isolates were found in seven out of eight trap plant sites although in one of these six sites, only one colony was found on all of the 150 plants. One isolate from these sites was found to contain the gene G143A. Barley trap plant results indicated that resistant powdery mildew colonies were found in all seven sites. Two isolates from these sites were found to contain the gene G143A.

The mean number of colonies per leaf was greater ($P < 0.05$) on unsprayed plants than on sprayed plants at all sites except at one wheat site. At this site, the mean number of colonies was significantly greater ($P = 0.007$) on the fungicide-treated leaves than on the untreated leaves. For wheat, mean numbers of colonies per leaf on unsprayed plants varied from below 1 to 27.5. For barley, mean numbers of colonies per leaf varied from below 1 to more than ten per leaf.

Four of the 15 *B. graminis* f. sp. *tritici* isolates and all 12 *B. graminis* f. sp. *hordei* isolates contained the gene G143A as indicated by PCR.

DISCUSSION

These results confirm that populations of *B. graminis* resistant to QoI fungicides are affecting wheat and barley crops in Canterbury. Isolates carrying the gene G143A express high (complete) resistance. As a consequence, continued applications of solo QoIs over time are very likely to result in severe loss in disease control. Further surveys of resistance to these fungicides have not been carried out since spring 2005. These should be carried out as soon as possible to monitor the situation so that growers can adopt appropriate fungicide resistance management strategies.

ACKNOWLEDGEMENTS

We thank Foundation for Arable Research for funding, and participating growers and Mr Grant Hagerty for assistance in these studies.

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Cross inoculation of crown rot and Fusarium head blight isolates of wheat

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INTRODUCTION

Fusarium head blight (FHB) and crown rot (CR) of wheat are two cereal diseases caused by Fusarium pathogens in Australia. Both *Fusarium graminearum* and *F. pseudograminearum* have been responsible for FHB epidemics (1, 2), while *F. pseudograminearum* is most commonly associated with CR. Recently, reports of *F. graminearum* causing CR have emerged from glasshouse seedling tests (3).

Crown rot and FHB are linked through aetiology, pathogen biology and epidemiology. The presence of perithecia on stubble could potentially allow the infection of wheat seedlings following ascospore release. Similarly, lodging of crops during heavy rain would allow wheat heads to come in close proximity of wheat crowns or residue with sporodochia of *F. pseudograminearum*, allowing FHB infection through splashed macroconidia (1). This does suggest however that there is no pressure for pathogenic specialisation as an individual isolate must retain pathogenicity for both crown and head infections.

While *F. graminearum* has been isolated from adult wheat crowns in the field, and can cause CR in a seedling test, evidence to demonstrate CR caused by *F. graminearum* in the field is lacking. It is hypothesised that while the fungus can invade wheat seedlings, it does not cause the typical symptoms associated with CR caused by *F. pseudograminearum*. To examine this, the relative pathogenicity of *F. graminearum* and *F. pseudograminearum* was compared using an adult CR pot assay against a selection of wheat genotypes.

With the potential of FHB caused by *F. pseudograminearum* occurring in fields with high levels of CR inoculum, it is necessary to understand the relative pathogenicity compared to *F. graminearum*, and the influence of FHB resistance on disease expression. This was achieved through screening a selection of wheat genotypes against both FHB pathogens.

MATERIALS AND METHODS

A selection of Australian varieties and breeding lines representing the range from resistant to susceptible for both FHB and CR were screened against *F. graminearum* FHB pathogenic and *F. pseudograminearum* CR pathogenic wild type cultures for CR. This was achieved by allowing 20 seedlings of each genotype to emerge and grow to the 3 leaf stage before adding sterile wheat bran to the surface of the pot and inoculating with a plug of agar containing the fungus. Disease severity was scored based on the extent of browning using a 0–4 scale at maturity.

The same selection of genotypes was also screened for resistance to FHB caused by both *F. graminearum* and *F. pseudograminearum*. An aliquot of 10µL of a 50000 spores.mL⁻¹ solution was pipetted into a single floret midway along the rachis of 40 heads for each genotype at anthesis. The inoculated heads were incubated for 48hrs at high humidity and a further 19 days under moderate humidity at 25°/18°C (day/night). The heads were scored visually by counting the number of affected grains in each head, measuring the level of spread of the pathogen.

RESULTS AND DISCUSSION

Results of the CR assay display a significant affect of genotype for both *F. graminearum* and *F. pseudograminearum* (P<0.01), suggesting that existing partial resistance is effective against both pathogens. Further, while *F. graminearum* is capable of causing stem browning in adult plants, the level of browning was significantly lower than that caused by *F. pseudograminearum* (P<0.01). However, the degree of stem browning did not differ significantly between *F. graminearum* and *F. pseudograminearum* for the three genotypes highly susceptible to CR; *Kukri*, *Kamilaroi* and *Batavia*. It is suggested that lines highly susceptible to CR may not be able to resist the invasion of even mildly pathogenic species.

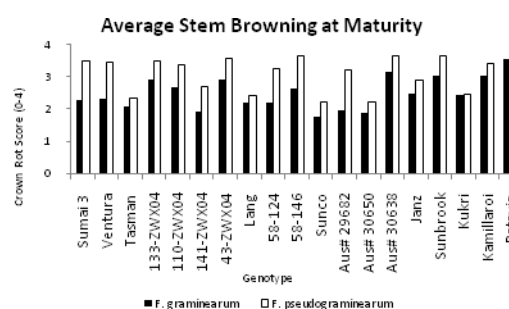


Figure 1. Results of CR scoring of both *F. graminearum* and *F. pseudograminearum* isolates.

Only preliminary data for the FHB assay is currently available. However, initial results suggest that the two pathogens have the same pattern of aggressiveness on genotypes with differing resistance. This supports previous studies which have demonstrated the non-specificity of FHB resistance using *F. culmorum* and *F. graminearum*.

This research has demonstrated the ability of *F. pseudograminearum* CR isolates to cause FHB and *F. graminearum* FHB isolates to cause basal browning, demonstrating a lack of specialisation of the pathogens in the field. The varying degree of basal browning caused by the two pathogens however suggests that differences in aggressiveness do exist. This should be considered when developing screening protocols.

ACKNOWLEDGEMENTS

The research was completed with the assistance of a GRDC Grains Research Scholarship.

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Twenty years of quarantine plant disease surveillance on the island of New Guinea: key discoveries for Australia and PNG

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INTRODUCTION

The island of New Guinea lies just 5 km from Australia's northern border and has a history of plant pest incursions, presumably from the west. Since 1989, quarantine plant pathologists from Papua New Guinea (PNG) and the Australian Quarantine and Inspection Service (AQIS) have conducted regular joint surveys in PNG's border regions. Since the mid 1990s, this search for exotic pathogens became annual. Less frequently over this period, Australian teams have also worked with Indonesian counterparts in the Indonesian province of Papua, which occupies the eastern half of the New Guinea land mass.

MATERIALS AND METHODS

Herbarium specimens and other kinds of plant tissue samples are collected, rendered quarantine secure, and returned under permit to various laboratories in Australia, PNG, and occasionally elsewhere, for diagnostic testing.

RESULTS

Major detections of quarantine significance are listed in Table 1. These are backed up by a list of finds of other important crop pathogens published in the last decade including first records on the island of New Guinea of *Banana streak GF virus*, *Banana streak Mys virus*, and *Banana streak OL virus*; first records in PNG of *Citrus tristeza virus*, *Papaya ringspot virus* –type W, *Watermelon mosaic virus*, and *Zucchini yellow mosaic virus*; first valid laboratory confirmation of *Fiji disease virus*, in sugarcane; and unpublished first records of *Cucumber mosaic virus* and teak rust caused by *Olivea tectonae* (P. Koko, NAQIA unpublished data, 2008).

DISCUSSION

These collaborative surveys have provided a rich source of quarantine incursion information for both Australia and PNG. Critical initial detections of major pathogen threats to

horticultural, field and ornamental commodities of importance to both countries have been made (Table 1). Moreover, the ongoing and regular nature of this work in PNG facilitated close monitoring of spread, following incursion, of fusarium wilt of banana and huanglongbing (HLB) of citrus. Whilst later finds of *Foc* VCG0126 were quite distant from the original one, HLB has been effectively contained since its discovery in 2002.

These surveys have also yielded critical information on the distribution of certain diseases endemic in PNG, but still of extreme quarantine concern to Australia. These include citrus canker, caused by *Xanthomonas citri* subsp. *citri*, and black Sigatoka disease of banana caused by *Mycosphaerella fijiensis*. Although citrus canker is widespread in the border regions, it is patchy in distribution: detected only in well separated larger communities. Black Sigatoka, in contrast, is ubiquitous, and over 100 samples have been collected.

In addition, certain key pathogens of regional quarantine concern, because of their recent history of spread in south east Asia, have not been detected on any AQIS surveys on the island of New Guinea. These include *Banana bunchy top virus*, cause of bunchy top disease of banana, and Cavendish competent strains of *Guignardia musae*, cause of freckle disease of banana. No convincing symptoms of bunchy top disease have ever been seen and all samples so far tested by enzyme linked immunosorbent assay were negative. Over 50 *G. musae* or *Phyllosticta musarum* (anamorph) herbarium specimens have been collected. However, all were from cooking bananas (ABB genome) or plantains (AAB genome). In the case of many of these collections, uninfected Cavendish sub group bananas were growing adjacent or nearby.

The results summarised here underline the critical importance, to Australia and PNG, of effective quarantine vigilance. This can only be achieved with an ongoing program of regular surveillance.

Table 1. Highlights of general disease surveys on the island of New Guinea, 1989–2009

Year	Location ^A	Host	Pathogen	Key discovery	Published ^B
1993	Papua, Indonesia	<i>Musa</i> sp.	<i>Fusarium oxysporum</i> f.sp. <i>cubense</i> (<i>Foc</i>) -VCG0126	First record of fusarium wilt of banana on the island of New Guinea	APP, 25
1996	SP, PNG	<i>Musa</i> sp.	<i>Foc</i> -VCG 0126	First record of fusarium wilt of banana in PNG	APP, 25
1997	Papua, Indonesia	<i>Musa</i> spp.	<i>Foc</i> -VCG 01213/16	First record of 'tropical race 4' of <i>Foc</i> on the island of New Guinea	APP, 29
1999	Papua, Indonesia	<i>Musa</i> sp.	Blood disease bacterium	First record of blood disease of banana on the island of New Guinea	APP, 29
1999	Papua, Indonesia	<i>Citrus</i> spp.	' <i>Candidatus</i> Liberibacter asiaticus'	First record of huanglongbing (greening disease) on the island of New Guinea	APP, 29
1999	Papua, Indonesia	<i>Oryza sativa</i>	<i>Rice tungro bacilliform virus</i>	First record of rice tungro disease on the island of New Guinea	APP, 29
1999	Papua, Indonesia	<i>Arachis hypogea</i>	<i>Bean common mosaic virus</i> , peanut stripe strain	Confirmation of widespread occurrence of peanut stripe disease in Papua	APP, 31
2002	SP, PNG	<i>Citrus</i> spp.	' <i>Ca. L. asiaticus</i> '	First record of HLB in PNG	APP, 33
2006	SP, PNG	<i>Heliconia</i> sp.	<i>Puccinia heliconiae</i>	First record in PNG	APDN, 3

^ASP: MP: Morobe Province, Sandaun Province, WP: Western Province

^BAPP: Australasian Plant pathology, APDN: Australasian Plant Disease Notes, volume number is listed.

The importance of reporting suspect exotic or emergency plant pests to your State

Department of Primary Industry

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INTRODUCTION

The Emergency Plant Pest Response Deed (EPPRD) (1) is a formal legally binding agreement between Plant Health Australia (PHA), the Australian Government, all State and Territory Governments and plant industry signatories covering the management and funding of eradication responses to Emergency Plant Pest (EPP) Incidents. Plant Health Australia is the Custodian of the EPPRD and it became operative on October 26, 2005.

The EPPRD replaces previous informal arrangements and provides a formal role for industry to participate and assume a greater responsibility in decision making in relation to EPP responses.

DISCUSSION

The EPPRD only operates for the eradication of EPPs. There are four criteria in the EPPRD for the definition of an EPP and the Pest only has to satisfy one of these to be considered an EPP. Briefly, these are:

1. A new pest to Australia
2. A different variation or strain of established pest
3. A previously unknown pest
4. A confined or contained pest

The formal definitions can be found in Clause 1.1 Definitions of the EPPRD. There is a list of EPPs that have already met one of the definitions and have been Categorised in Schedule 13 of the EPPRD.

For an eradication response to be agreed it must be both technically feasible and cost beneficial to eradicate the pest. As such, early reporting of suspect emergency plant pests is a critical step in the process. The longer it takes for a suspected EPP to be reported, the more time the pest has to become established and more wide spread. This increases the costs of containment, control and eradication measures, reduces the technical feasibility and therefore reduces the likelihood of success of eradication. Figure 1 demonstrates the differences in probability of successful eradication compared to time from detection to reporting.

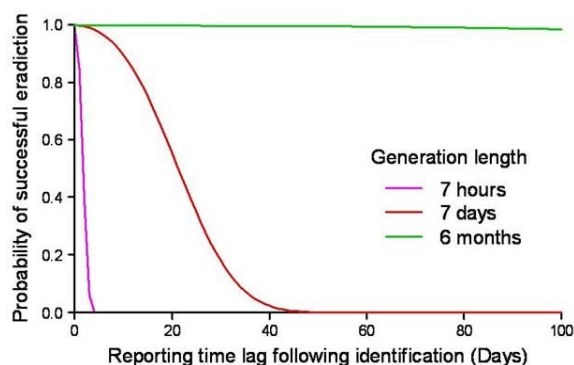


Figure 1. Probability of successful eradication compared to reporting time lag following identification.

Government Signatories to the EPPRD undertake to provide formal notification within 24 hours of becoming aware of an incident and take all reasonable steps to ensure that persons within their jurisdiction are aware they need to advise that government within 24 hours of becoming aware of an incident so that the formal notification can be made. It is important that diagnosticians and researchers understand their responsibility, not only a moral obligation to protect Australian agriculture and horticulture but this legal obligation that now exists for jurisdictions and their personnel. Personnel of government agricultural agencies need to report a 'reasonably held suspicion' of an exotic pest to their jurisdiction's Chief Plant Health Manager directly or via the Exotic Plant Pest Hotline (1800 084 881).

It is also important for the integrity of the EPPRD that all Parties (government and industry) understand their rights and responsibilities and adhere to them. If Parties do not adhere, as closely as possible, to their responsibilities under the EPPRD, the work done to improve collaboration and trust between all Parties is eroded.

Many significant plant pests are cryptic and not readily visible. Red Imported Fire Ant is estimated to have been present for as many as five years before detection, likewise European House Borer may have been present for as long as 50 years before detection.

Both of these pests would have cost considerable less to eradicate if they had been detected and reported within the first few generations.

Not only does the EPPRD provide an obligation to report a 'reasonably held suspicion' but there is the potential for cost sharing of actions taken to be rejected if it is deemed that there has been a failure to report in a timely manner.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Peter Caley of the Bureau of Rural Sciences, Department of Agriculture, Fisheries and Forestry for his assistance with generating the graphical data.

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The use of sentinel plantings in forest biosecurity; results from mixed eucalypt species trails in South-East Asia and Australia

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INTRODUCTION

Many diseases of *Eucalyptus* species have emerged as pathogens in exotic plantations. Guava rust (*Puccinia psidii*), cryphonectria canker (*Crysothecium cubensis*), coniothecium canker (*Colletogloeopsis zuluensis*) and Kirramyces leaf blight (*Kirramyces destructans*) are all serious pathogens that have not been found in native forests or in plantations in Australia (Burgess & Wingfield 2002; Cortinas *et al.* 2006; Glen *et al.* 2007; Wingfield *et al.* 2001). The susceptibility to these pathogens of *Eucalyptus* spp. commonly used in exotic plantations is known; however the susceptibility of many *Eucalyptus* spp. found only in natural ecosystems in Australia is unknown. There are two main uses of sentinel plantations. Firstly, tree species known to be susceptible to different pathogens can be planted within the natural environment to try and trap pathogens from their surroundings. In Australia, taxa trials planted in different environments act as sentinel plantings. By surveying these taxa trials we have collected and described a number of new eucalypt pathogens and reported the presence in Australia of *Kirramyces destructans*. The second use for sentinel planting is where many tree species are planted in a region known to harbour certain pathogens. In this manner the susceptibility of the different tree species can be determined.

MATERIALS AND METHODS

Taxa trials and adjoining natural vegetations have been surveyed in tropical and sub-tropical Australia. This involves collection of diseased leaf and canker material, isolating the fungus using standard techniques and identification of the fungi using classic taxonomy and molecular phylogeny

We have established sentinel trials of 25 eucalypt species in Vietnam, China and Thailand in regions known to harbour *Kirramyces destructans* and *Colletogloeopsis zuluensis*. To date only the trial in Vietnam has been surveyed for impact of leaf pathogens and insects. In addition, trees have been inoculated with *Colletogloeopsis zuluensis* and lesion formation and lesion length measured. A matching trial has also been planted in northern Australia.

RESULTS AND DISCUSSION

We have focused our sampling in northern Australia on fungi causing disease; on leaves the Mycosphaerellaceae predominated, especially *Kirramyces* species, the dominant pathogens in cankers belong to the Botryosphaeriaceae. Many of the species found have been described on eucalypts either in Australia, but often elsewhere where eucalypts are grown. Several new fungal species have been described. In the trial in Northern Australia only eucalypt species already known to be susceptible to *K. destructans* developed Kirramyces leaf Blight. The other 20 eucalypt species did not develop symptoms

The trials in Vietnam have been monitored with the main finding being an expansion of the host range of the gall wasp *Leptocybe invasa* and the leaf pathogen *Quambalaria eucalypti* and the discovery of a new *Quambalaria* sp (Figure 1). The greatest lesion length after inoculation with *Colletogloeopsis zuluensis* was observed for *Eucalyptus saligna* and *E. pellita*, two species

not previously known to be affected. The trials in China and Thailand will be assessed in July 2009 prior and results available prior to the APPS conference in October

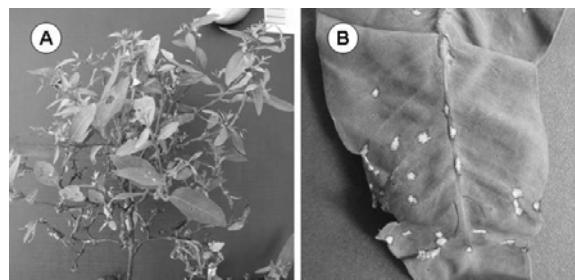


Figure 1. (A) *Leptocybe* damage on young petioles, (B) *Quambalaria* spp. forming white spots on leaves

These sentinel trials, established in Asia, will provide valuable information on the susceptibility to some of the keystone tropical *Eucalyptus* spp. to various exotic pathogens.

ACKNOWLEDGEMENTS

The work was supported by an ARC Discovery project DP0664334. We thank Great Southern Limited for provided land and maintaining trial on the Tiwi Islands.

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Methyl bromide alternatives for quarantine and pre-shipment and other purposes—future perspectives

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INTRODUCTION

The switch to methyl bromide alternatives for fumigation purposes has largely hinged on the ozone depleting status of this chemical.

This paper explores the major plant and plant product commodities treated with methyl bromide for quarantine and pre-shipment use on an Australian and international basis.

The underlying domestic and international policy context, technical, regulatory, legislative, market access and trade and environmental factors, are all key drivers or impediments to adoption of alternatives.

Yet there is no 'silver bullet' to replace methyl bromide treatment, or is there? Or is a paradigm shift required with how quarantine risk is assessed or when, how or if treatments are applied?

Some of the key alternatives for plant and plant product commodities are identified, as are the underlying issues that determine the adoption or otherwise of alternatives.

MATERIALS AND METHODS

The Plant Health Committee consisting of plant protection officers from the Australian government and state biosecurity agencies commissioned an investigation of what methyl bromide alternative treatments were available for quarantine and pre-shipment and other plant uses. Specifically, what alternatives were under research, under registration and or in use in Australia and/or in use and/or registered internationally.

To ensure data accessibility and an ongoing commitment to evaluating alternatives, an information system, the Methyl Bromide Alternatives Information System was created. International Plant Protection Convention standards for commodity classification and acceptance of a new phytosanitary treatment were adopted to ensure that the dataset would meet national and international requirements.

Whilst collating data on alternatives, a series of themes emerged. Through this process the major drivers and impediments to adopting alternatives were identified.

RESULTS AND DISCUSSION

The efficacy of alternative chemical or non-chemical methods of fumigation was found not to be the primary driver for the switch to a methyl bromide alternative.

Instead the underlying context of consumer demand for chemical free alternative treatments, occupational and environmental health concerns, more intense scrutiny for chemical registration and the European Union's decision to deregister methyl bromide from March 2010 were the most significant contributing factors for adopting methyl bromide alternatives.

The time between research and commercialisation of alternatives, the requirements for registration and variance in data protection laws contributed to different countries adoption

of alternatives. Adoption by a country of alternatives is also dependent on quarantine services approving a treatment for use, and for trade using the alternatives being acceptable to both importing and exporting countries.

The Methyl Bromide Alternatives Information System was designed to capture these factors and to provide a transparent and internationally acceptable repository for data on alternatives.

The Methyl Bromide Alternatives Information System has over 150 registered users and contains over 480 records. Initially registration was limited to within Australia. Since December 2008 membership has been expanded to international subscribers through the IPPC network and by inviting further data input from the Quads countries—United States, Canada and New Zealand.

Alternatives will be discussed using a case study of Australia's highest volume uses for QPS and the pros and cons of alternatives that have been identified.

Pests of quarantine concern where no efficacious alternatives have been identified, or for which there are no approved Australian quarantine treatments other than methyl bromide will also be discussed.

The presentation will include a live demonstration of MB AIS and conference delegates will be encouraged to become registered users and data contributors to the system.

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www.daff.gov.au/mbais

Fruit extracts of *Azadirachta indica* induces systemic acquired resistance in tomato against *Pseudomonas syringae* pv *tomato*

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INTRODUCTION

Last couple of decades has witnessed a spurt in use of plant extracts as biocontrol agents for controlling a wide range of crop pathogens. However mechanism of action of such extracts has not been well understood.

MATERIALS AND METHODS

In the present study 6 week old plants of two cultivars of tomato i.e. wild and a F1 hybrid were treated with aqueous fruit extracts of *A.indica* (neem). The treated plants were inoculated with spores of *Ps Syringae* either 24 hrs prior to or after neem treatment. Leaf samples were collected from inoculated non-inoculated treated and control plants at intervals of 24 hours for 6 days, for estimating the activity of phenylalanine ammonia lyase, tyrosine ammonia lyase, polyphenol oxidase and contents of total phenol, mRNA and proteins. Disease intensity was recorded after 3rd and 10th week of inoculation. Neem treated and control plants were also treated with inhibitors of transcription and translation.

RESULTS AND DISCUSSION

Wild cultivar after treatment has substantially lower infection after neem treatment as compared to F 1 hybrid and controls. In both cultivars there was a sharp increase in activity of PAL, TAL, PPO and concentration of total phenols. Treated plants had new mRNA and high concentration of some proteins. Treatment with inhibitors of transcription resulted in absence of new mRNA and reduced concentration of some proteins. Translational inhibitors inhibited the increase in concentration of proteins. SDS-PAGE analysis had revealed increased concentration of PAL, TAL, PPO. Disease incidence of neem treated plants after 10 weeks of inoculation was substantially less than control. Results reveal that neem extracts control pathogen on tomato through induction of SAR. SAR induction is a function of plant genome as evidenced from difference in results of wild and F1 hybrids.

Fungal foliar endophytes induce systemic protection in cacao seedlings against *Phytophthora palmivora*

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INTRODUCTION

The fungal endophytes of cacao (*Theobroma cacao*) comprise a diverse assemblage including pathogens and saprophytes. The interaction between these endophytes and their host is not yet understood, although some fungal endophytes may protect their host against pathogens¹.

Black pod, caused by *Phytophthora* spp. is responsible for losses to global cocoa production of around 20-30% annually². In Papua New Guinea, the main methods of controlling black pod caused by *P. palmivora* are to use resistant cultivars and cultural practices, as fungicides have limited efficacy and are generally not cost-effective for small-holder farmers. Biological control of cocoa diseases using endophytic fungi may provide another tool in the Integrated Pest and Disease Management toolbox. The purpose of this study was to identify common fungal endophytes present in Australia and Papua New Guinea capable of reducing disease severity of disease caused by *P. palmivora*. Such knowledge is an essential first step in the development of a biological control agent and will help to solve unanswered questions regarding the ecological role of fungal foliar endophytes.

MATERIALS AND METHODS

Endophytes were sampled from leaves and pods of cacao growing in five locations in Australia and Papua New Guinea. Common endophyte taxa were screened for the ability to reduce the growth of *P. palmivora* *in vitro* via dual culture and pre-colonised plate assays. The same endophyte taxa were tested for the ability to reduce severity of foliar disease caused by *P. palmivora*. Cacao seeds were germinated and either exposed to ambient endophyte spores in the glasshouse or were kept endophyte free by raising seedlings in sterile soil in a clean room. Two leaves on each seedling were infected with a 5.6×10^6 propagules/ml suspension of a single endophyte taxon or of all taxa combined. Endophyte infection was tested 17 days later by harvesting one leaf from each seedling, surface sterilising leaf fragments and then plating them onto growth media. 18 days after endophyte inoculation, one endophyte infected and one endophyte free leaf on each seedling was inoculated with *P. palmivora* by applying a 30µl drop of zoospore suspension (400,000 zoospores /ml) to the leaf midvein which had been pierced with a sterile hypodermic needles. Control seedlings were mock inoculated with sterile water. Ten seedling replicates were prepared for each treatment and control. Disease severity was measured five days later as the length of the necrotic lesion along the mid vein.

RESULTS

Five of the common endophyte taxa tested inhibited the growth of *P. palmivora* when co-cultured on corn meal agar compared to *P. palmivora* grown alone. Subsequent experiments showed that one *Xylaria* morphotype inhibited the growth of *P. palmivora* via antibiosis whilst the other endophytes were inhibitory via competition for resources. Two endophyte taxa that were inhibitory *in vitro*, as well as three other common endophyte taxa reduced disease severity in seedlings that had been grown in an endophyte free environment. Additionally,

inoculation of one cacao leaf on a seedling with either *Phomopsis* or *Diplodia* resulted in uninoculated leaves on the same plant being less susceptible to disease compared to completely endophyte free seedlings. In contrast, addition of endophyte inoculum to seedlings that had already been infected with ambient endophytes in the glasshouse did not result in a reduction in disease severity caused by *P. palmivora*.

DISCUSSION

Results from these experiments demonstrate that common, non-coevolved endophyte taxa can increase the resistance of their host plants to pathogens. In some cases, endophyte mediated protection is systemic and therefore likely to be due to induction of a non-specific defence response in the host. Harnessing this apparently beneficial interaction between endophytes and their host to manage plant disease may not be easily achievable as our results show that addition of more endophytes to already infected seedlings did not decrease disease severity. However, these results suggest that fungal foliar endophytes have a protective interaction with their host in a broader ecological sense.

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Effectiveness of the rust *Puccinia myrsiphylli* in reducing populations of the invasive plant bridal creeper in Australia

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INTRODUCTION

Bridal creeper (*Asparagus asparagoides*) is a dense scrambling vine that smothers large areas of vegetation and threatens native biodiversity in Australia (1). In winter rainfall areas, it begins to grow in late summer – early autumn, produces fruit in mid to late spring and above-ground foliage naturally senesces at the beginning of summer. Bridal creeper has been the target of a biological control program since the 1990s. Three agents of South African origin have since been released: the leafhopper *Zygina* sp. in 1999, the rust fungus *Puccinia myrsiphylli* in 2000 and the leaf beetle *Crioceris* sp. in 2002 (2). Both the leafhopper and rust fungus have established widely on bridal creeper populations across temperate Australia. The rust fungus however, is the most effective agent, probably due to its major indirect impact on the plant's below-ground biomass (3).

We report on results from two different approaches used to measure the effectiveness of *P. myrsiphylli* in reducing populations of bridal creeper across Australia.

MATERIALS AND METHODS

Before and after release comparisons. One to three years before releasing the rust, support structures (or trellises—2 m in height and 90 cm wide) were set up at the edge of 3 m² plots (three to four plots per site) in bridal creeper infestations at 15 sites across southern Australia. Growth and reproductive parameters of bridal creeper in a 1 m² quadrat within each plot and climbing on the trellis were recorded in mid-spring each year for up to 8 years after the release. Incidence of the rust was also measured annually. This long-term experiment was performed in partnership with a range of collaborators across Australia.

Fungicide exclusion experiments. Permanent 1 m² quadrats (10 per site) with a central 100 cm long stake were set up in bridal creeper infestations at three sites in NSW and three sites in WA, where the rust fungus was the dominant agent. Sites in WA were managed by CSIRO Entomology staff based in Perth. Half of the quadrats at each site were maintained rust-free using monthly fungicide applications during the growing season for 4 years. The remaining quadrats were sprayed with water only. Percentage cover of bridal creeper and other plant species within quadrats, as well as other bridal creeper growth and reproductive parameters, and disease incidence and severity were measured each year.

RESULTS

Before and after release comparisons. After the release of the rust, bridal creeper seedling and shoot numbers and above-ground biomass in the permanent 1 m² quadrats steadily declined at all sites (Fig. 1). In contrast, the impact of the rust on bridal creeper climbing onto trellises varied considerably between sites.

Fungicide exclusion experiments. Bridal creeper cover, above-ground biomass, and shoot, fruit and seedling numbers were substantially lower in rust-infected compared to rust-free quadrats across all sites over the years. The cover of bare ground and leaf litter was consistently higher in rust-infected quadrats than in rust-free quadrats. When other plants did establish in

rust-infected quadrats, bridal creeper was replaced by both native and exotic species, although other weeds were more prevalent at disturbed sites.

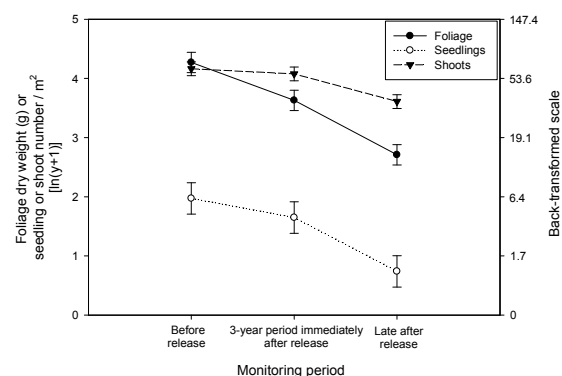


Figure 1. Changes in bridal creeper growth parameters in permanent 1 m² quadrat established at 15 sites across southern Australia and monitored 1–2 year before and up to 8 years after the release of *P. myrsiphylli*.

DISCUSSION

These studies demonstrate that *P. myrsiphylli* has major negative impacts on bridal creeper populations, although some sites may need to be carefully managed to facilitate native species recovery. For example, only a few plant species were present in rust-infected quadrats by the end of the 4-year fungicide exclusion experiment. More than four consecutive seasons of severe rust epidemics on bridal creeper may thus be required to sufficiently reduce populations and trigger major recruitment by other plants.

ACKNOWLEDGEMENTS

We gratefully acknowledge the contribution to these experiments of our collaborators from The Departments of Primary Industries in NSW and Victoria, The Department of Water, Land and Biodiversity Conservation in South Australia and CSIRO Entomology based in Canberra and Perth. We also wish to thank Bob Forrester of CSIRO Entomology for his assistance with statistical analyses. This work was supported by CSIRO, Weeds CRC, the Australian Government (NHT & Defeating the Weed Menace initiative) and the respective organisation of our collaborators.

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Evaluation of essential oils and other plant extracts for control of soilborne pathogens of vegetable crops

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INTRODUCTION

Soilborne plant pathogens including *Pythium* spp, *Fusarium* spp, and *Rhizoctonia* spp, can cause important diseases such as root rot and damping off, resulting in heavy crop losses in vegetable farms. Control of these diseases is problematic because these pathogens have a wide host range and survive in soil as oospores, chlamydospores and melanised hyphae, respectively, for long periods. Compounds derived from plant extracts have been proposed as potential control treatments for soilborne pathogens due to their antimicrobial activity in laboratory studies (1). For instance, essential oils can contain phenolic and terpenoid compounds which have antimicrobial properties (2). The antimicrobial activity of thyme oil has been shown to cause hyphal collapse by membrane disruption (1). In Australia, very few studies have investigated the effects of antimicrobial volatile compounds in essential oils on survival of soilborne pathogens. Our work is therefore investigating the antimicrobial activity of compounds derived from a range of essential oils and other plant extracts against key soilborne pathogens isolated from vegetable crops. Preliminary results from *in vitro* experiments are reported here.

MATERIALS AND METHODS

A series of *in vitro* experiments were conducted to investigate the effects of plant extracts on mycelial growth of soilborne pathogen isolates. Treatments tested included the active constituents (eugenol, thymol, carvacrol and geraniol) of some essential oils as well as 14 essential oils (thyme, clove bud, peppermint, geranium, eucalyptus, tea tree, origanum, rosemary, orange sweet, cardamon, sweet fennel, pine, black pepper and basil). The pathogenic isolates tested included *Pythium sulcatum*, *P. aphanidermatum*, *P. irregulare*, *Fusarium oxysporum* and a *Rhizoctonia* sp. Solutions of the actives and the oils were added to sterile suitable selective media at concentrations of 500, 1000, and 2500 ppm. A 5 mm mycelial plug was then plated onto the amended media. Plates with *Pythium* were incubated at 20°C and those with *Fusarium* and *Rhizoctonia* at room temperature. Mycelial growth, expressed as colony diameter, was measured until mycelium in unamended plates reached the edge of the plate. After this, plugs that did not grow were transferred to fresh unamended media to determine whether the treatments were fungistatic or fungicidal. Examples of results are given for some of the isolates tested to illustrate the suppressive and biocidal effects of some of the oil treatments tested.

RESULTS

All concentrations of the four plant actives significantly ($p < 0.001$) inhibited mycelial growth of all isolates tested. Mycelial plugs of these isolates did not regrow once transferred to fresh media. Treatments containing the essential oils thyme, origanum and clove bud also significantly inhibited mycelium ($p < 0.001$) at all concentrations and mycelium did not regrow on fresh media. However, other oil treatments varied, with some only suppressing mycelial growth at higher concentrations. For instance, 2500 ppm geranium, peppermint and pine oils significantly ($p < 0.001$) reduced mycelial growth of *F. oxysporum* and *Rhizoctonia* sp., compared to unamended controls (Table 1).

This effect was also observed for *P. aphanidermatum*, *P. sulcatum* and *P. irregulare*. Geranium oil completely inhibited growth of *F. oxysporum* and *Rhizoctonia* sp. at 1000 and 2500 ppm (Table 1), and growth did not resume on fresh media. Pine oil completely inhibited *Rhizoctonia* sp. at 1000 ppm and 2500 ppm (Table 1), but mycelial growth resumed at 1000 ppm. Basil, cardamon, sweet fennel and tea tree oils only suppressed growth at the highest concentration tested, and mycelial growth resumed on fresh media. Rosemary, eucalyptus, orange sweet and black pepper oils did not inhibit mycelial growth compared to the unamended plates.

Table 1. Effects of three oil treatments on mycelial growth *in vitro*.

Oil/Pathogen	% inhibition of mycelial growth ¹		
	500 ppm	1000 ppm	2500 ppm
Pine oil			
<i>F. oxysporum</i>	26.4	79.3	100 ²
<i>Rhizoctonia</i> sp.	69.7	100	100 ²
Peppermint oil			
<i>F. oxysporum</i>	20.3	35	65.3
<i>Rhizoctonia</i> sp.	0	45.3	94.1 ²
Geranium oil			
<i>F. oxysporum</i>	55.9	100 ²	100 ²
<i>Rhizoctonia</i> sp.	43.5	100 ²	100 ²

¹% inhibition compared to unamended controls at 10 days.

²No mycelial growth resumed after transfer to fresh media.

DISCUSSION

Preliminary *in vitro* results indicate that four plant extract actives and three essential oils were biocidal to mycelium of some isolates of soilborne pathogens from vegetable crops. Other oil treatments only delayed mycelial growth but did not affect mycelium viability, or had no effect on mycelial growth at all. The biocidal activity observed by origanum, clove bud and thyme oils against *Pythium* spp, *Fusarium oxysporum* and *Rhizoctonia* sp. is likely to be due to specific chemical compounds contained in these oils. For instance, carvacrol and thymol, also found to have biocidal effects, are major components of origanum and thyme oils respectively (2). Future experiments will determine which compounds and dosages are the most effective at killing mycelium and other resting structures of pathogens. The mechanisms by which these compounds affect inoculum viability will be investigated to better understand their mode of action so they can be developed further for disease control.

ACKNOWLEDGEMENTS

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

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Use of grid weather forecast data to predict rice blast development in Korea

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Timely warnings on plant disease development are useful information for farmers to determine when to spray fungicides to control plant diseases. Real-time weather data monitored by automated weather stations are often used to generate disease forecast information. However, when observed weather data are used, the time window for effective fungicide sprays after the disease warnings could be too short and farmers may fail to control the disease in time even though accurate disease forecast is available. In order to minimise the time limitations associated with real-time disease forecasting, the weather forecast data need to be used for disease forecasting. At every 3 hours, the Korea Meteorological Administration (KMA) releases 48-hour weather forecasts at 3-hour intervals on air temperature, relative humidity, and probability of precipitation and at 12-hour intervals on precipitation based on the outputs from numerical weather prediction models. The spatial resolution of weather forecasts is 5 km. Using the grid weather forecasts, rice blast disease forecasting was conducted. The grid forecast data at 3 hour intervals are interpolated to produce hourly data. Hourly wetness period was estimated from a simple relative humidity model and a CART model using temperature, relative humidity, precipitation, and wind speed. Based on the hourly weather data, daily risk levels of rice blast infection were determined at the spatial resolution of 5 km in the map image. The results suggested that estimation of hourly leaf wetness needs to be improved to enhance the accuracy in forecasting infection periods.

Investigating the impact of climate change on plant diseases

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INTRODUCTION

Climate change is recognised as a major threat to agricultural systems and will likely alter the risks associated with the biosecurity and market access of its agricultural products (1). The potential effects on pest and disease threats to these changing systems are not well understood. Specifically, there is a critical lack of empirical data on how increasing atmospheric carbon dioxide (CO₂) will impact on pest and pathogen populations and crop production. Our approach to investigate the impact of climate change on disease threats is two-fold. We developed models to analyse vector-borne diseases of a number of endemic and exotic diseases and their hosts. Further, we have embarked upon a real time field study of the effects of elevated (e)CO₂ on pathogens of wheat, using the Free-Air CO₂ Enrichment (FACE).

METHODS

Modelling. We used a bioclimatic model (CLIMEX) to investigate the potential distribution of citrus canker (*Xanthomonas citri* pv *citri*). A second modelling approach (using STELLA) combined dynamic sub-models of host-plant physiology, vector population growth and climatic data to investigate the effect of climate change on the exotic Asiatic citrus psyllid (*Diaphorina citri*) which vectors the citrus disease huanglongbing (citrus greening). This approach was also used to develop an integrative model for the bird cherry-oat aphid (*Rhopalosiphum padi*) under increasing temperatures which vectors Barley yellow dwarf virus in wheat.

FACE. Three wheat pathogens were targeted to examine the influence of CO₂ on their biology and interaction with their host, *Puccinia striiformis* (wheat stripe rust), *Fusarium pseudograminearum* (crown rot) and *Barley yellow dwarf virus (BYDV)*. Wheat stripe rust severity, latent period, fecundity and host resistance was assessed under ambient and 550ppm CO₂. Crown rot severity on Tamaroi (very susceptible variety) and 2–49 (a partially resistant breeding line variety) were also assessed. The RPV strain of *BYDV* was isolated and maintained in oat and perennial ryegrass by serial aphid transfer using *R.padi* and transmitted to wheat growing under eCO₂. DNA analysis of soil from ambient (aCO₂) and eCO₂ plots for soil-borne pathogen detection was also completed.

RESULTS

Modelling. CLIMEX results revealed that the predicted citrus canker distribution would shift to southern coastal and inland regions under increasing temperatures, based on its current climatic range overseas. The STELLA model indicated earlier, shorter development times and a population shift southwards for the potentially invasive Asiatic citrus psyllid (*Diaphorina citri*), under increasing temperatures. However, an overall decrease in psyllid numbers is predicted due to reduce availability of new growth flushes on which the psyllid feeds and reproduces (2).

FACE. There was no significant effect of eCO₂ on stripe rust progress, latent period, fecundity and disease resistance but a significant increase in the severity of crown rot symptoms and fungal biomass under eCO₂. The methodology for analysing *BYDV* movement in wheat under eCO₂ has been established. In 2007, visual scoring of *BYDV* symptom expression between eCO₂ and aCO₂ treatments showed no significant difference. The 2008 *BYDV* inoculation results are being analysed.

DISCUSSION

Modelling tools are available to predict geographic ranges of plants and their pests and pathogens but often do not take into consideration the specific biology of the organisms and host-pathogen/pest interactions. The value of this approach is confirmed by the Asiatic citrus psyllid model which would have only predicted shorter development times and an increase risk of potential distribution based on climatic data alone. Such improved models could be incorporated into plant disease management plans and contingency planning. The predictive power of the models will be increased with real-time data generated by the FACE experiments. Limited chamber and field studies have shown an increase in aggressiveness of some fungal pathogens and enhanced host susceptibility (3,4) which was supported an increase severity of crown rot symptoms and fungal biomass under eCO₂. For wheat stripe rust, cultivar resistance and seasonal rainfall have more significant effects on disease progress than increased atmospheric (550ppm) CO₂. Further analysis is required to make an assessment of eCO₂ effects on *BYDV* titre and movement in wheat and aphid vectoring capacity.

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Impact of climate change in relation to blackleg on oilseed rape and blackspot on field pea in Western Australia

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INTRODUCTION

Worldwide, including Western Australia (WA), blackleg and blackspot are the most important diseases affecting production of oilseed rape (or canola) and field pea, respectively. Synchronisation of ascospore release with the seedling stage often results in particularly severe canker formation and correspondingly major yield losses for oilseed rape. Therefore, delay in onset of release of ascospores until the susceptible seedling stage has passed could be beneficial to oilseed rape. In contrast, field pea crops are susceptible to blackspot throughout all growth stages, therefore, it is recommended that growers delay crop establishment to avoid the peak of release of ascospores. In order to estimate the impacts of climate change on the severity of these diseases, it is important to identify the potential shifts in the pattern(s) of ascospore release for these diseases under the anticipated future climate conditions.

METHODS

The 'Blackleg Sporacle' model was used to determine the timing of onset of release of blackleg ascospores and the 'Blackspot Manager' model was used to estimate the peak of release of blackspot ascospores in eight locations across the grain-belt of WA, viz., Badgingarra, Corrigin, Dalwallinu, Esperance, Lake Grace, Merredin, Wagin, and Wandering. The models were run with statistically downscaled CSIRO Mk3 GCM simulation weather data based on the IPCC SRES A2 emission scenario (2036–2060) (1). This future scenario was compared with recorded climate data for the period of 1976–2004 inclusive.

Table 1. Predicted timing of onset of blackleg ascospore release under current and future climates. DOY denotes day of the year (as of Julian day, 1 being 1 January and 366 being 31 December).

Location	Onset of ascospore release		
	Current (DOY)	Future (DOY)	Difference (day)
Badgingarra	170	181	11
Corrigin	168	179	11
Dalwallinu	183	196	13
Esperance	104	116	12
Lake Grace	163	177	14
Merredin	177	191	14
Wagin	150	168	18
Wandering	149	164	15
Average	158	172	13

RESULTS AND CONCLUSIONS

Model runs with these climate scenarios show that, on average, the onset of release of blackleg ascospores is likely to be delayed by about two weeks (Table 1). Whereas, the opening seasonal rains (also known as 'break of the season') were delayed by about one week (data not shown). Hence, the onset of blackleg ascospores, relative to the break of the season, will occur only one week later than currently. Therefore, the risk of synchronisation of major blackleg ascospore showers with seedling establishment appears to be similar under future climates. The predicted future increase in temperature may

slightly favour canker formation in oilseed rape (data not shown), with the potential for ideal conditions for canker formation becoming more frequent.

On the contrary, the peak of the release of blackspot ascospores is predicted to be about four weeks earlier than under current climatic conditions; relative to break of the season, the release of blackspot ascospores will take place in about five weeks earlier (Fig. 1). That scenario would be highly beneficial to field pea growers, allowing earlier sowing dates to reap the greater agronomic yield potentials while being exposed to relatively low blackspot disease pressure.

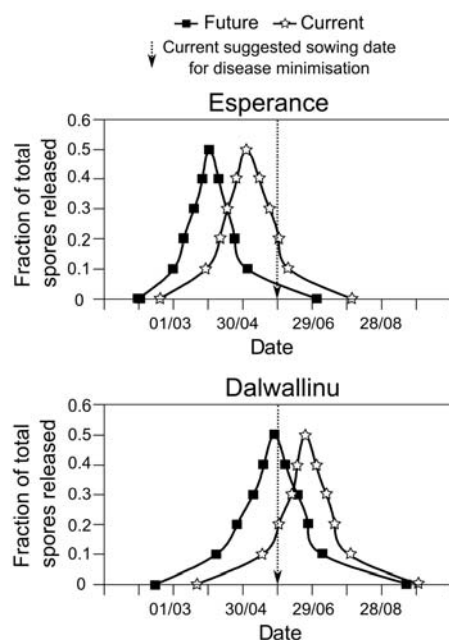


Figure 1. Predicted temporal pattern of blackspot ascospore release under current and future climate Esperance and Dalwallinu regions of Western Australia.

ACKNOWLEDGEMENTS

We thank the Australian Grains Research and Development Corporation (GRDC) and the Department of Agriculture and Food Western Australia for supporting this research.

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Approaches to training in plant pathology capacity building projects in developing countries

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Plant diseases continue to be an impediment to the socio-economic progress of developing countries. The ability of plant pathologists in these countries to accurately diagnose and provide advice on integrated disease management is often limited by an overall low level of training and lack of diagnostic specialists in country. Laboratory facilities are frequently inadequate and ongoing access to consumable laboratory materials and maintenance of equipment is difficult due to limited financial resources.

Australia is an active donor to agricultural capacity building projects in many developing countries through government agencies and other funding bodies. Because capacity building projects focus on sustainable development through skills transfer, training of counterparts in developing countries is an inherent component of any capacity building project. Here we outline what we consider to be the three most important elements of training where many capacity building projects can fail: 'English in Context' training, suitability of Australian postgraduate courses and extended periods of in-country training. Our recommendations are based on our experience working on plant pathology capacity building projects in Vietnam, Indonesia, Laos, China and Tunisia over the past 15 years.

'English in Context' training. Language can be a barrier to effective training for counterparts from non-English speaking countries. A level of sufficiency in technical English used in plant pathology is required in order for counterparts to communicate with Australian mentors and interpret for international visitors, access internet resources and seek information from literature and disease compendia. 'English in Context' refers to the use of English as a second language in the workplace. We recommend that all capacity building projects in non-English speaking countries include an intensive 'English in Context' training component at the outset with ongoing training provided through the life of the project. This approach has been used to great effect in Vietnam (1).

Suitability of Australian postgraduate courses. Many counterparts aim to obtain scholarships to undertake postgraduate training in Australia in association with capacity building projects. Modern Australian plant pathology courses are becoming increasingly focused on molecular biology, with a decline in classical diagnostic training. However, in developing countries the greatest need is for field and laboratory training in basic plant pathology skills. Providing students from developing countries with advanced training in molecular techniques is illogical and ill conceived. Identification of a pathogen to species level or below is often not necessary for effective integrated disease management practices to be implemented. Supervisors need to consider the suitability of coursework undertaken by students more carefully as well as their ability to continue mentoring the student on their return.

In-country training. For a more complete understanding of plant diseases, it is important for counterparts to follow the full diagnostic process from field surveys, through isolation and identification of a pathogen, followed by pathogenicity testing to complete Koch's postulates. This requires the availability of the facilitator or mentor to be in-country for extended periods of time. Although this is often difficult, it can be more cost effective than holding short workshops in Australia and can potentially reach more counterparts. The presence of a mentor or facilitator in country allows real-life examples currently affecting farmers to be used in training programs. It also forces an acknowledgement of local diseases, agronomic practices and laboratory conditions by the facilitator. We also recommend a focus on interactive teaching, encouraging student participation. This approach facilitates 'learning by doing'.

The Australian Youth Ambassador for Development (AYAD) program is an ideal program for placing skilled young Australians in developing countries for up to 12 months. We encourage project leaders to consider including an AYAD in their project plan and for plant pathology graduates to seek such assignments.

ACKNOWLEDGEMENTS

We gratefully acknowledge funding from the Australian Centre for International Agricultural Research and the ATSE Crawford Fund as well as the contribution of our many colleagues with whom we have collaborated over the last 15 years, both in Australia and in developing countries.

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Increasing global regulations on fumigants stimulates new era for plant protection and biosecurity

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INTRODUCTION

Restrictions on fumigant chemicals due to environmental concerns (eg. Montreal Protocol) and tropospheric pollution (eg. new volatile organic compound regulations) has caused a surge in new strategies to control soilborne pathogens, but are sustainable practices being adopted? Since the early 1990's when methyl bromide (MB) was shown to be responsible for ozone layer degradation, a massive global research effort has been undertaken to find alternative disinfestation strategies. It was anticipated that growers would readily adopt practices which conserved biodiversity and the principles of biological equilibrium. In reality this has not happened because pathologists have not yet developed a sufficient understanding of the relationships between soil biology and plant yield or the mechanisms of disease suppression in the absence of synthetic chemicals.

After MB phase out, many sectors still use other fumigant chemicals (Fig 1), as other technologies have not always provided the same advantages afforded by soil fumigation, (i.e. a high level of pest and disease control with low risk, and good quality and high yielding crops). However, a bigger set of factors is now influencing crop production and crop protection; climate change, user and bystander safety, increasing prices of water, oil and inorganic fertilisers and concern over soil health is finally being recognised. This is causing a shift in grower approaches to soilborne disease control in crop protection.

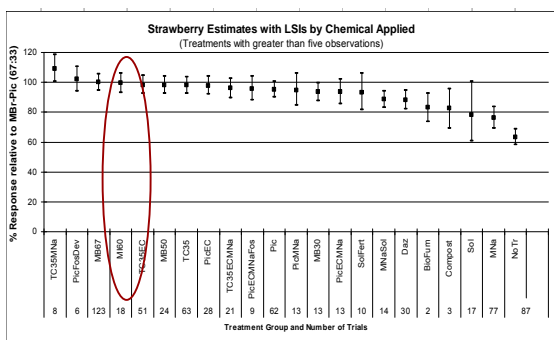


Figure 1. The efficacy relative to MB/Pic (67:33) of a large number of crop protection practices on yield of strawberry plants in a metaanalysis of over 100 international studies (1). Ellipse - MB/Pic standard

Also, whilst new strategies are being developed for sustainable control of soilborne pathogens very little attention is being given to products or strategies which eradicate soilborne pathogens and protect industries from incursions from exotic pathogens. In fact, there is a poor knowledge on the ability of any strategies to effectively eradicate soilborne microorganisms. Techniques, such as solarisation and steaming whilst effective on a limited scale are not totally effective in the field, biofumigants decrease risk but do not eradicate propagules and chemical fumigants do not guarantee total eradication. Development of soilless systems which exclude soilborne pathogens are a key future practice to eliminate disease, and use of grafting and resistant varieties can reduce effects of disease (Table 1).

Table 1. Some of the future technologies that will be relied upon to replace control of soilborne plant pathogens by chemical fumigants C: - commodity treatments

Present Disinfestants	IPM Strategies to replace disinfestants
Telone C35 EC	Grafting and plant resistance
Chloropicrin EC	Biorationals (AG3, Voom)
Methyl iodide	Biofumigants
Dimethyl disulphide	Endophytes?
Solarisation	Biocontrols?
Streaming	Strategic pesticides and herbicides
C:Sulphuryl fluoride	Soilless systems: Substrates and hydroponics
C: phosphine/CO ₂	MB Recapture and recycling

IMPACT OF FUMIGANT USE FOR BIOSECURITY

Fumigation of imported and exported commodities is a key activity for quarantine and pre-shipment especially to satisfy phytosanitary requirements of the importing country. MB is the key fumigant used for QPS and is presently exempt from phase out under the rules of the Montreal Protocol, although the European Community has decided to phase out all uses by March 2010. Although MB is predominantly used to eradicate insect pests from commodities, it may not have any significant control of fungal pathogens, however this is not clearly understood. A key alternative for QPS commodity treatments, sulphuryl fluoride, also has concerns for use as it has a very high global warming potential (GWP ~ 4000). Therefore the key to successful QPS treatments for Australia would be to maintain a systems approach which only allows imports from regions where the diseases (eg. *Phytophthora ramorum*, Guava rust, etc.) are not known to occur.

The Australian and international scientific community require answers to some key questions to minimise the impact of changes to availability of fumigants. For instance, in the future, will we have strategies to eradicate soilborne pathogens in the event of an incursion? Is it worth the investment to try to eradicate a soilborne organism? Why is MB being retained for use in nursery industries worldwide where high health is paramount, when its fungicidal properties may be insufficient? Has Australia identified the major exotic soilborne (and airborne) pathogen risks? Is Australia really prepared to cope with an outbreak of a serious exotic soilborne pathogen?

This paper will further discuss some of the future challenges facing industries when considering adoption of new bioprotection, biosecurity or crop management practices.

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A world of possibilities: the importance of international linkages

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The issues facing our societies and professions are global in nature. Plant pathology as a discipline is no different since plant pathogens know no geographic or political boundaries. Through the efforts of individual scientists, global networks were established for scientific disciplines and this has been occurring through the ages. Many of our professional societies even when national based have international members indicating the importance of international networks. The American Phytopathological Society began their efforts in international programs in the 1940s and refined their goals in 1983 and again in 2005. However these efforts did not result in international linkages that bring together scientific societies. For there to be linkages across societies there must be assurance of each society being successful and that ventures undertaken across societies must complement each other's strengths and fill in potential weaknesses. Linkages must seek common goals and interests. Possible items that could generate linkages across our societies will be explored. In order to move forward in the 21st century, it is time to embrace networks of alliances to advance the knowledgebase and this requires international linkages.

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21 First report of *Leveillula taurica* on *Ficus carica* (matrix nova)

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INTRODUCTION

Leveillula taurica Lev. Belong to Erysiphaceae (Erysiphales, Ascomycota) that causing powdery mildew on over from 50 plant families(1).

MATERIALS AND METHODS

During the summer 2007, typical symptoms of powdery mildew were observed in several fig fields assessed in Kereman Province, Iran. Samples were stained with Lactofushin(3) and morphological characteristics of fungus investigated by Olympus microscope (Modle: BH2) and drawn by drawing tube connected on microscope.

RESULTS AND DISCUSSION

Morphological characters of this fungus on *ficus carica* is as follow:

Diseased leaves displayed typical powdery mildew signs consisting of whitish masses of conidia and conidiophores. Mycelial growth was thick, forming irregulare white patches, sometimes effused to cover the whole leaf surface and usually not present appressoria. Conidiofores erect, foot cells cylindric, 40–126 (-148) × 4/5–7/8 μm usually followed by (1-) 2–3 (-4) shorter and different length cells. Conidia formed singly, primery conidia lancaeolate, 31–67 (80) × 12–20 μm and secondary conidia ellipsoid to cylandric, 33–76 × 13–22 μm (fig 1). Ascomata found on leaves as embedded in the mycelial felt, were gregarious to scattered and measured 145–250 μm in diameter. Appendages were myceliod, arising from the lower half of ascomata, brown, paler upward. Asci 20–30 (-45) in each cleistothecia, clvate, stalked, 77–120 (-135) × 25–42 μm. Ascospores (1-) 2 (-4) in each ascus, ellipsoid-ovoid shaped, (20-) 25–40 (-45) × 15–22 μm (fig 2).

On the basis of morphological characters of the anamorph and telemorph, this fungus was identified as *Leveillula taurica* (1). This is also the first report of genus *Leveillula* on Morceae in world and Morceae is a new host family for *Leveillula taurica*(1,2).

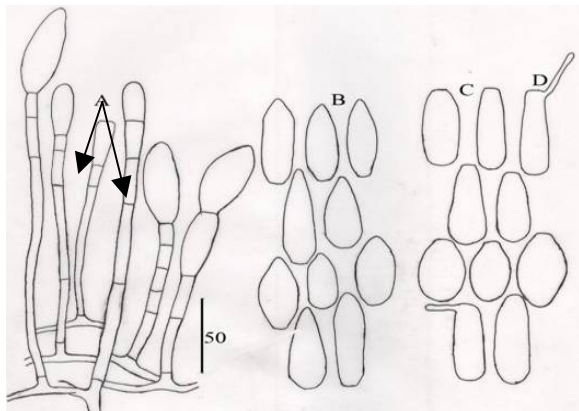


Figure 1. *Leveillula taurica* (anamorph). (A) conidiophores, (B) primery conidia, (C) secondary conidia, (D) germinated conidium.

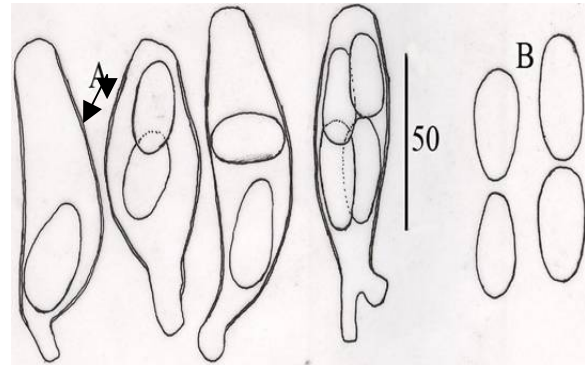


Figure 2. *Leveillula taurica* (telemorph). (A) asci, (B) ascospores.

The fungus caused significant losses, estimated at more than 35% of production, at the location studied by making infected plants unsuitable for use in propagation.

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57 New records of Erysiphaceae (Ascomycota: Erysiphales) for Iran mycoflora

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INTRODUCTION

Powdery mildew fungi belong to the family Erysiphaceae (Ascomycota: Erysiphales) and infect a wide range of angiosperms. This family consists of 18 genera and about 435 species(1). Ershad (4) has provided the best list of powdery mildews and their hosts in Iran.

MATERIALS AND METHODS

During the years 2007 and 2008 Surveys were carried out to determine species composition and host range of powdery mildews(Erysiphaceae) in Sistan region, Iran. Samples were stained with Lactofushin(3) and morphological characteristics of fungus investigated by Olympus microscope(Modle: BH2) and drawn by drawing tube connected on microscope. Observation of conidial germ tubes was carried out using the method of Hirata (5). Identification of species were carried out by reliable references(1,2).

RESULTS AND DISCUSSION

In this study fourteen taxa were identified which according Ershad(4) Among this taxa, *Erysiphe australiana* is new to Iran mycoflora and other thirteen taxa viz. *Erysiphe convolvuli*, *E. cruciferarum*, *E. lycopsidis*, *E. necator*, *E. polygoni*, *Golovinomyces cichoracearum*, *G. orontii*, *Leveillula saxaouli*, *L. taurica*, *Phyllactinia moricola*, *Podosphaera leucotricha*, *P. pannosa* and *Blumeria graminis* have previously been recorded from Iran.

Morphological characters of *Erysiphe australiana* on *Lagerstromia indica* is as follow:

Mycelium on leaves, effused, cover the whole leaves surface, nonpersistence, appressoria are multilobed. Conidiofores erect, foot cells cylindric occasionally ruffle, (23-) 25–36 (-50) (5-) 6×-9/5 μm followed by (1-) 2 (-3) different length cells. Conidia formed singly, ellipsoid to cylindric, (25-) 28–43 (-49) × (11/5-) 12/48–18 (19/5) μm. This fungus didn't form teleomorph(fig 1).

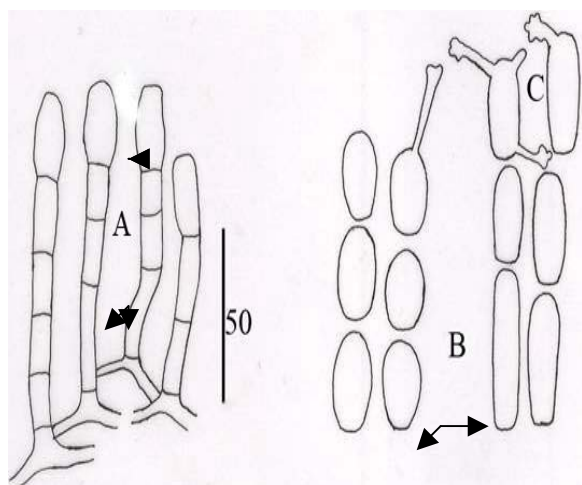


Figure 1. *Erysiphe australiana* on *Lagerstromia indica*: A—conidiophores, B—conidia, C—appressoria

Morphology of appressoria, conidiophores, conidia as well as the host species, fit the description for *Erysiphe(Uncinuliella) australiana* provided by Braun (1). conidia with multilobed appressoria and the host species determined that *Oidium yeneii* donot occurs on *Lagerstromia indica*.

So, for your more information also six plant species viz. *Alhagi persarum*(a host for *L. taurica*), *Althaea officinalis*(a host for *L. taurica*), *Gaillardia* sp.(a host for *G. cichoracearum*), *Haloxylon persicum*(a host for *L. saxaouli*), *Malva microcarpa*(a host for *L. taurica*) and *Phalaris paradoxa*(a host for *B. graminis*) are recorded as a new hosts for Iranian powdery mildew fungi.

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58 Survey of propinquity among *Erysiphe*, *Leveillula*, *Phyllactinia*, *Podosphaera*, *Sphaerotheca*, *Uncinula* and *Uncinuliella* based on analysis of morphological characters

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INTRODUCTION

Powdery mildew fungi belong to the family Erysiphaceae (Ascomycota: Erysiphales) which cause serious diseases in a variety of cultivated plants such as cereals, vegetables and ornamental plants. This family consists of 18 genera and about 435 species (1).

Phylogenetic relationships among the genera of powdery mildews have been proposed by some authors (1, 2, 3).

This research is carried out in order to investigate propinquity and affinity among seven genera of powdery mildews based on morphological data.

MATERIALS AND METHODS

We used 18 morphological characters. Each character contains 2 to 6 status. Varies status of characters take numbers 1 to 6. The data were analyzed using the Distance method by PAUP v.4.0b4a (4). Neighbour-Joining (NJ) tree was obtained. The strength of the internal branches from the resulting trees were tested by bootstrap analysis (5).

RESULTS AND DISCUSSION

The results showed that all taxa are divided into five groups, which corresponded well to new mitosporic taxa. Clade 1 consisted of *Erysiphe* section *Erysiphe*, *Uncinula* and *Uncinuliella*, all of which have single conidia, lobed apparatus and an *Oidium* subgenus *Pseudoidium* mitosporic state. Clade 2 consisted of *E. galeopsidis*, *E. cichoracearum* and *E. orontii*, which have without fibrous bodies catenate conidia and *Oidium* subgenera *Striatooidium* and *Reticulooidium* mitosporic states. Clade 3 consisted of *Leveillula* and *Phyllactinia*, which have endophytic mycelia and same surface patterns of conidia and *Oidiopsis* and *Ovulariopsis* mitosporic states, respectively. Clade 4 consisted of *Podosphaera* and *Sphaerotheca*, which have fibrous bodies catenate conidia and single asexual ascocarps and an *Oidium* subgenus *Fibroidium* mitosporic state. Clade 5 consisted of *Blumeria graminis*, which has digitate haustoria, bulbous swelling of foot cell, unique surface patterns of conidia and an *Oidium* subgenus *Oidium* mitosporic state. This result coincides with molecular analyses (1,3)

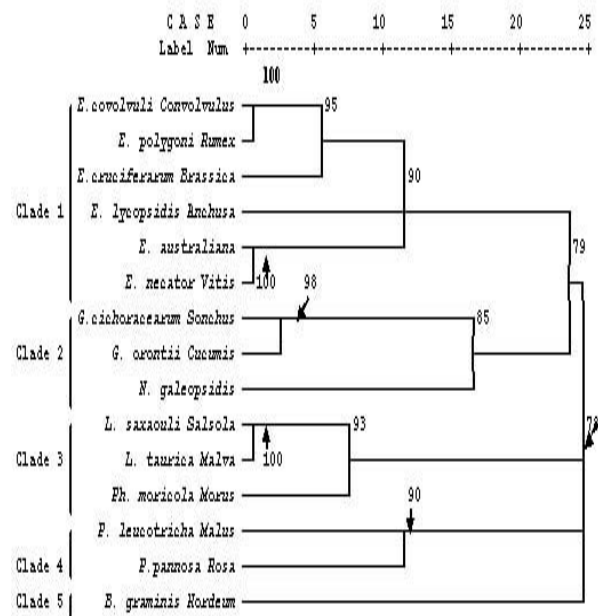


Figure 1. Neighbour-Joining tree inferred from morphological data. Branch support was determined by 1000 bootstrap replication, shown above the branches. Bootstrap values below 50% are not shown.

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22 Pulse virus surveys from Victoria and South Australia in 2007

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INTRODUCTION

Surveys of pulse crops in 2007 (field pea, faba bean, lentil, lupin and chickpea) were undertaken in Victoria and South Australia to determine the occurrence and incidence of eight pulse viruses: *Alfalfa mosaic virus* (AMV), *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Pea seedborne mosaic virus* (PSbMV), *Bean leafroll virus* (BLRV), *Beet western yellows virus* (BWYV), *Tomato spotted wilt virus* (TSWV) and *Subterranean clover stunt virus* (SCSV).

MATERIALS AND METHODS

In Victoria in October 2007, random samples were taken from 45 crops, comprising 13 field pea, eight lentil, 15 faba bean, five chickpea and four lupin from 31 locations (Ararat, Berriwillock, Boyeo, Clear lake, Culgoa, Dimboola, Dooen, Douglas, Gymbowen, Inverleigh, Jung, Kaniva, Kerang, Lake Boga, Lalbert, Linton, Marnoo, Minimay, Mininera, Minyip, Murtoa, Natimuk, Nhill, Noradjuha, Rockwood, Rupanyup, Skipton, Tresco, Warracknabeal, Woorinen and Ultima). In South Australia in October 2007, random samples were taken from 49 crops, comprising 20 faba bean, 11 field pea, nine lentil, two chickpea, five lupin and two vetch from 27 locations (Agery, Arthurton, Blyth, Bordertown, Brecon, Cockle Beach, Coonalpyn, Cummins, Freeling, Giles Corner, Glen Park, Hart, Karkoo, Keith, Kybunga, Minlaton, Monta, Mundulla, Owen, Rhynie, Riverton, Rogers Corner, Tarlee, Saddleworth, Warooka, Willalooka, Yeelanna). One hundred, randomly selected petioles, tendrils or shoots were collected from each crop and bundled into groups of ten. The bundles were blotted onto nitrocellulose membranes and processed using tissue blot immunoassay. Within-crop virus incidence for each crop was estimated from the number of positive samples.

RESULTS

In Victoria, the most serious virus problems were BWYV in pea, lentil and chickpea, CMV in lentil and PSbMV in pea (Table 1). BWYV occurred in 100% of pea and chickpea and 88% of lentil and 67% of bean crops and the within crop virus incidence was highest in lentils (up to 61%) and lowest in beans (up to 8%). CMV occurred in all crop types with low within crop incidence (<11%) except in lentils where the virus incidence ranged from 5–100% and 50% of lentil crops were infected. PSbMV was only a problem in peas with 38% of crops infected and the within crop incidence ranged from 2–90%. AMV, BYMV, BLRV and SCSV infected less frequently with lower within crop incidence except for BYMV in pea (up to 24%) and SCSV in bean (up to 28%). TSWV was only detected from pea with low incidence.

Results in South Australia, were similar to Victoria, with BWYV and CMV generally causing the greatest virus problems with PSbMV a problem in peas (Table 2). BWYV occurred in 90% of bean and in 100% of pea, lentil and chickpea crops and the within crop incidence was highest in pea (up to 79%) and lowest in chickpea (up to 25%). CMV occurred in all crop types, but the within crop virus incidence was up to 100% in lentils and lupins and 100% of lentil and 40% of lupin crops were infected. PSbMV was only a problem in peas with 45% of crops infected and the within crop virus incidence ranged from 1–74%. The within crop virus incidence of AMV, BYMV and BLRV was generally low. SCSV

only occurred in some crops and at low within crop incidence in bean and TSWV not detected at all.

DISCUSSION

In Victoria, the within crop virus incidence of CMV in lentils, BWYV in pea, lentil and chickpea, PSbMV in peas and SCSV in bean were all very high compared to previous years (1). Virus infected seed may have been the source of BYMV, CMV and PSbMV. In South Australia, CMV is an ongoing problem in lentils and lupins (2). The other major seedborne virus in SA was PSbMV in peas. It is thought that both of these viruses originated from infected seed. The severity of BWYV was significantly greater in bean, pea, lentil and chickpea in both states than in previous years (2). BWYV has been more of a problem under drought conditions and aphid vectors appear to be leaving alternate hosts such as medic and lucerne perennial pastures and feeding on young pulse crops.

Table 1. Summary of Victorian pulse survey; number of infected crops (% virus incidence)

No crops sampled	Bean 15	Pea 13	Lentil 8	Chickpea 5	Lupin 4
AMV	0	3 (2–13)	5 (3–13)	2 (2–9)	1 (2)
BYMV	3(1–7)	4(1–24)	0	0	3(5–11)
CMV	1(1)	1 (1)	4 (5–100)	3(1–10)	1 (9)
PSBMV	0	5(2–90)	0	0	0
BLRV	1(2)	8 (1–5)	3 (1–2)	0	0
BWYV	10(1–8)	13 (8–36)	7 (5–39)	5 (18–61)	0
SCSV	6(1–28)	1(2)	0	2(5–6)	0
TSWV	0	2(1–6)	0	0	0

Table 2. Summary SA pulse survey; number of infected crops (% virus incidence)

No crops sampled	Bean 20	Pea 11	Lentil 9	Chickpea 2	Lupin 5
AMV	1(2)	0	4(3–17)	1 (4)	1 (12)
BYMV	1(1)	2(2)	3(1–4)	0	0
CMV	2(2–4)	2 (2–41)	9(4–100)	2(3–10)	2 (25–100)
PSBMV	2 (3–5)	5(1–74)	1(4)	0	0
BLRV	5(3–10)	3 (1–3)	2(4–6)	0	0
BWYV	18(1–57)	11(3–79)	9(17–73)	2 (8–25)	0
SCSV	6(1–6)	0	0	0	0

Note: All samples were negative to TSWV.

ACKNOWLEDGEMENTS

This research was funded by GRDC (Project DAV 0004188). The authors thank Shane. King and Chris Wilmshurst for assistance.

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24 Integrated management of mango diseases using inoculum reduction strategies with fungicide spray treatments

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INTRODUCTION

Anthracnose caused by *Colletotrichum gloeosporoides* (Penz.) and stem-end-rots caused by *Neofusicoccum parvum* (*Botryosphaeria* spp and *Lasiodiplodia theobromae*) are the main postharvest diseases of mango in all tropical and sub-tropical environments where mangoes are grown.

Managing these diseases effectively is the key for producing quality mango fruits with a long shelf life. The use of pre- and post-harvest fungicide treatments has been the main mechanism of trying to achieve this objective (1). There are environmental and residue concerns on the overuse of these fungicides and therefore a push to limit their use. To do this, other strategies need to be integrated with minimal fungicide use to overcome such concerns and still achieve effective disease control. The main objective of this study was to determine the effectiveness of integrating field inoculum reduction strategies with minimal fungicide sprays in managing mango postharvest rots, especially anthracnose and stem end.

MATERIALS AND METHODS

The trials were conducted on a uniform block of 18-year old Kensington Pride mango trees at Ayr Research Station of the Queensland Primary Industries and Fisheries, during 2007 and 2008 seasons. The block was divided into two sub-blocks to represent partial and optimal inoculum reduction levels. On the partial reduction sub-block a one-time removal of dead twigs, branches and leaves from the tree canopy was undertaken soon after mechanical pruning, while on the optimal reduction sub-block dead twigs, branches and leaves were removed from within and underneath the trees soon after pruning, and were followed up with monthly repeats of the same exercise.

The following fungicides were applied to the treatment trees in different combinations at strategic times in each season: Mankocide (Mc), Mancozeb (Mz), Octave (Oc), Amistar (Am), Bravo (Br), Aero (Ae) and Tilt (Tt). This was to determine their integrated effects with the inoculum reduction strategies on mango postharvest diseases. The treatment trees in each sub-block were arranged in a 6 x 4 RCBD and standard mango industry tree husbandry practices for irrigation, fertilisation and insect pest control were implemented.

At harvest, 35 fruits were randomly picked from each treatment tree from which 25 more uniform ones were selected, de-seppped, washed and then placed in boxes and stored in a cool room at ~20–22°C. Fruits were assessed for postharvest rots disease incidence 14 days after incubation.

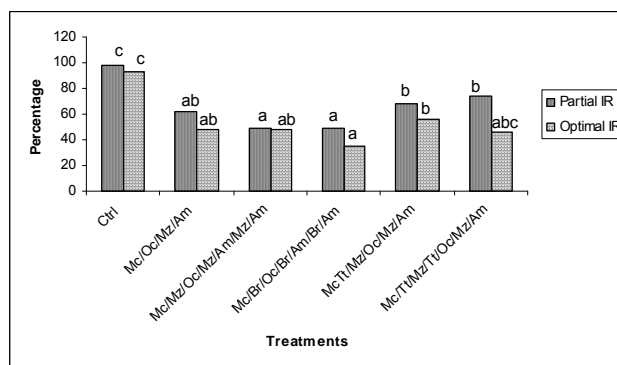
RESULTS AND DISCUSSION

All fungicide spray combinations in 2007 and 2008 were significantly ($P=0.05$) better than the control in suppressing postharvest rots incidence on the fruits (Figs 1 and 2). In 2008 there were additional treatment differences within the fungicide treatment combinations (Fig. 2). Significant differences ($P=0.05$) between partial and optimal inoculum reductions on fruit rots were observed on most treatments in 2008 but not 2007. The repeat of the inoculum reduction exercise on the same sub-block for two seasons significantly reduced the level of inoculum-

carrying dead materials within and underneath the treatment trees resulting in this accumulated significant effect in 2008.

Fungicide treatment combinations used in both seasons ranged from a minimum of 3 to a maximum of 7 sprays. This was significantly less than the current industry practice of up to 12 or more sprays per season, to achieve the same level of disease control as compared to the low levels from the optimal inoculum reduction sub-block.

These trial results demonstrate the role that basic orchard hygiene can play in field management of mango postharvest diseases, especially when integrated with minimal fungicide spray treatments.



{Ctrl = Control, Mc = Mankocide, Oc = Octave, Mz = Mancozeb, Am = Amistar, Br = Bravo, Tt = Tilt }

Figure 1. Effect of inoculum reduction (IR) and limited fungicide sprays on incidence of postharvest rots—2007

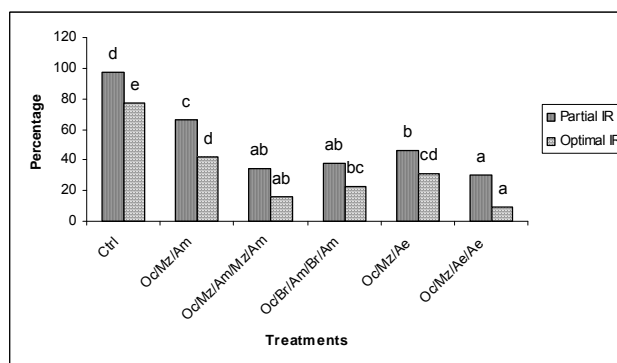


Figure 2. Effect of inoculum reduction (IR) and limited fungicide sprays on incidence of postharvest rots—2008.

ACKNOWLEDGEMENTS

We gratefully acknowledge HAL, AMIA and DPI&F for funding this research.

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23 Mango sudden death syndrome assessment in various mango growing districts of Punjab, Pakistan

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INTRODUCTION

Mango is an important fruit in Pakistan enjoyed by all age groups in the country. The continuous production of this fruit is being threatened by a number of diseases. Among these is the mango sudden death syndrome (MSDS), a complex caused by a number of biotic and abiotic factors. *Botryodiplodia theobromae* and *Ceratocystis fimbriata* have been identified as the key fungal pathogens in Pakistan that lead to an increase in the severity of the disease. Typical symptoms of this disease include drooping and browning of leaves, bark splitting, gum oozing, and in most cases these symptoms are followed by the sudden wilting and death of the tree.

The disease was first detected in Pakistan in 1995 from the Muzaffar Garh district of Punjab and its effects only became popularised in 2005 when it was reported to be spreading at epidemic proportions with serious effects on productivity (1). There has been a dire need to monitor the prevalence of the disease and identify factors associated with its rapid spread through out the mango production districts of Pakistan. The main objective of this study was to monitor the spread and distribution of the disease and collect data associated with its epidemiology as a first step towards the development of management strategies to stop or slow down its spread.

MATERIALS AND METHODS

A survey was conducted in the following mango growing districts of Punjab: Faisalabad, Jhang, Khanewal, Multan and Muzaffar Garh. The number of orchards visited in these districts was 3, 6, 3, 8 and 4 respectively. Sampling was done from the twigs, branches, bark, stem at the collar regions and roots of the affected trees. The assessment was made on the basis of a mean disease severity rating of 0–5 reflecting the percentage of symptoms such as gummosis, bark splitting and bark beetle holes observed on the parts sampled, where 0=healthy trees; 1=1–10%; 2=11–20%; 3=21–30%; 4=31–50% and 5= more than 50% diseased area.

The infected samples were cut into small pieces with a sterilised scissors and disinfected with 10% commercial bleach for one minute followed by three rinses in distilled water. After drying, the pieces were aseptically plated on potato dextrose agar medium and incubated at 25°C. After 7 days of incubation resulting fungal colonies were microscopically identified based on spore morphological characteristics. The colonisation frequency of each sample was also determined using the following formula:

$$\text{Colonisation (\%)} = \frac{\text{No. of pieces colonised by a pathogen}}{\text{Total No. of pieces}} \times 100$$

RESULTS AND DISCUSSION

The maximum of 27% mean disease incidence was found in Multan samples followed by 22% in Muzaffar Garh, 18% in Jhang and 15% in Khanewal. The minimum of 12% disease incidence was found in Faisalabad. A maximum severity rating of 4 was also observed on Multan samples followed by a 3 rating for both Khanewal and Muzaffar Garh and a 2 rating for Jhang samples. A

minimum rating of 2 was recorded on Faisalabad samples (Fig 1). These results clearly demonstrate that mango sudden death disease prevails in all the mango growing districts of Punjab surveyed. The common prevalence of the disease may be associated with the large number of abandoned orchards that are receiving little or no management attention. It was also common to observe adjacent orchards with high levels of infection, especially in the Multan district, suggesting the ease of pathogen movement between such orchards. The colonisation percentage of individual fungi from the different orchards sampled shows that *B. theobromae* was the most common pathogen while *C. fimbriata* was the least (Fig 2) This survey results suggest the need for more emphasis on the importance of orchard sanitation and improved cultural practices to reduce the prevalence of different fungal pathogens causing sudden death of mango in Punjab

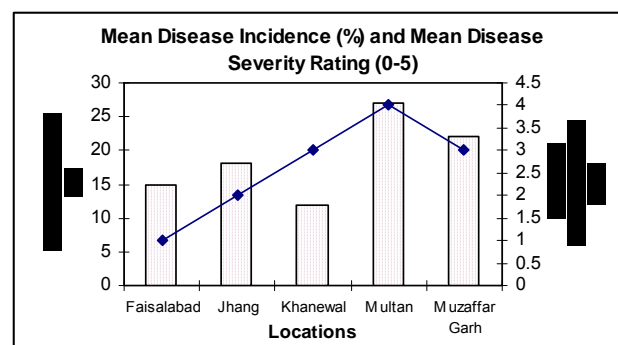


Figure 1. Mean incidence and severity of sudden death in different districts of Punjab.

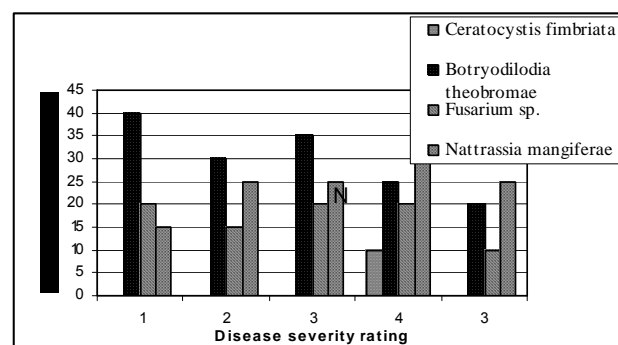


Figure 2. Mean disease incidence of different fungi colonising mango trees with sudden death symptoms in Punjab

ACKNOWLEDGEMENTS

Funding for this work was provided by Etiology and Management of Mango Project and ASLP Mango Production Project.

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1 Identification and characterisation of phytoplasma pathogen associated with alfalfa diseases in Al Hasa, Saudi Arabia

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INTRODUCTION

Alfalfa (*Medicago sativa* L.) is cultivated as a forage crop in many countries and is distinguished from other agricultural crops in having a perennial habit. However, phytoplasmas diseases have been reported to cause very significant economic losses in several countries worldwide. In Eastern province Saudi Arabia, alfalfa is the most important forage crop.

A number of phytoplasma diseases have been reported to be associated with alfalfa plants that result from drastic reduction in forage yield. Average annual loss of alfalfa in the Sultanate of Oman due to witches'-broom disease is approximately 25% of green hay, an estimated loss of US\$30 million (1).

Recently a phytoplasma of 16SrI, *Ca. Phytoplasma asteris*, has been associated with the Al-Wijam disease of date palm disease, in Al Hasa, Saudi Arabia, and leafhoppers belonging to *Cicadellidae* family were identified as potential vectors (2). This paper reports results of a PCR-based phytoplasma survey on diseased alfalfa grown in Eastern province of Saudi Arabia.

MATERIALS AND METHODS

Field-collection of plants, leafhoppers Alfalfa (*M. sativa* L.) surveys were conducted from April to September 2008 in Eastern province of Saudi Arabia (Fig. 1). A total of 76 samples showing typical symptoms of witches' broom disease. 254 leafhoppers were collected from alfalfa field. Type specimens were identified at the National Museum of Wales, Cardiff as follows: *Empoasca decipiens* (Paoli) and *Cicadulina bipunctata* (Melichar).

PCR and RFLP analysis DNA was extracted from leaf tissue and insects. Aliquots of final DNA preparations were used as template for a nested PCR (nPCR) assay with phytoplasma 16S rDNA primers R16mF2/R16mR1 for the first round, and either R16F2n/R16R2 and fU5/rU3 for the nested reaction. Nested PCR products (10 ml) were digested with restriction endonucleases AluI, HpaII, Hae III and Sau3A I.

16S rDNA sequencing and phylogenetic analysis Phytoplasma rDNA amplified by PCR using the primer pair P1/P7 was purified. The PCR products were sequenced in both directions using primer pair P1/P7 and the 16S rDNA sequences of phytoplasmas identified in our study were compared with others in Genbank by BLAST.

RESULTS AND DISCUSSION

Crop samples showing typical symptoms of phytoplasma infection were collected from different areas of Al Hasa (Fig 1). Leafhoppers were also trapped by netting for examination as potential vectors of the disease. Plant samples and leaf hoppers were analysed by DNA extraction and amplification with phytoplasma-specific primers.

Phytoplasmas were detected in 43/76 alfalfa samples, and from 16/33 batches of all leafhopper species tested. No PCR products were obtained for asymptomatic plant samples. RFLPs were used to partially characterise isolates from plants and insects. Based on RFLP and sequencing analysis, phytoplasmas from group

16SrII. The 16SrII group was found in alfalfa and all insect species tested. In the Gulf region, the 16SrII group has been identified in lime alfalfa (1).

Amplified DNA was sequenced to determine relationships between phytoplasma isolates. The work confirmed that phytoplasmas are infecting alfalfa crops in Saudi Arabia, and progress was made in identifying the phytoplasma groups present and information gained on their potential spread by vectors.

E. decipiens and *C. bipunctata*, the most abundant insects collected from fields, were carrying phytoplasmas from 16SrI and 16SrII in proportions of 10:33, and 6:33, respectively. Results suggest that *E. decipiens* and *C. bipunctata*, are the major insect candidates to vector phytoplasmas from 16SrI and 16SrII groups. It is known that many vectors can transmit more than one type of phytoplasma and that many plants can harbour two or more distinct phytoplasmas. Vector-host-plant interactions play an important role in determining the spread of phytoplasmas. It is very likely that due to their abundance and capability to carry phytoplasmas, *E. decipiens* and *C. bipunctata* mainly contributed to the spread phytoplasma diseases, in alfalfa, date palm, decline in lime and the disease in papaya, by cycling from the alternative reservoirs to the crops, so that, the spread of diseases is a consequence of the vector-phytoplasma-plant three way interaction.



Figure 1. Alfalfa witches'-broom symptoms

ACKNOWLEDGEMENTS

We thank BAE Systems and the British Council for funding the Post doctoral Summer Research Program for Dr Khalid Alhudaib. We thank Dr Mike Wilson at the national Museum of Wales, Cardiff, UK, for the finer identification of the leafhopper species.

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59 Efficient transformation of *Colletotrichum capsici*, the causal agent of chilli pepper anthracnose by *Agrobacterium*

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INTRODUCTION

Anthrachnose disease of chilli pepper is caused by a complex of *Colletotrichum* spp. with *C. capsici* being the most severe in South East Asia (1). Knowledge of the mechanisms for host resistance and pathogenicity is crucial for developing effective and durable disease control. Several putative pathogenicity genes involved in *C. capsici* infection of chilli pepper have been identified and partially cloned (Auyong, unpublished). A fungal transformation system is required to prove the function of these putative genes in the infection process. In this study an efficient transformation system was successfully developed to serve as a platform towards understanding chilli pepper-*C. capsici* interactions. *Agrobacterium tumefaciens* carrying a hygromycin phosphotransferase gene (*hph*) and a green fluorescent protein (*GFP*) gene was used to transform the conidiospores of *C. capsici*. Transformation efficiency was correlated with conidiospores density, ratio of conidiospores to bacterial cells, type of *Agrobacterium* strains and plasmid, presence or absence of acetosyringone, co-cultivation time and co-cultivation temperature.

MATERIALS AND METHODS

Fungal culture. *Colletotrichum capsici*, BRIP 26974 isolated from *Capsicum annuum* was supplied by the Department of Primary Industry (DPI), Queensland, Australia, and maintained on potato dextrose agar (PDA).

Fungal transformation. Conidial suspension was prepared and adjusted to 10^2 , 10^4 , 10^6 and 10^8 conidiospores per ml and mixed at different ratio (1:3, 1:5, 1:1, 3:1 and 5:1) with *Agrobacterium* (AGL1 or LBA4404) containing either pJF1, pPK2 or pKHt plasmid. The mixture was plated onto filter paper on solid induction medium, either amended or non-amended with 200 μ M acetosyringone. Following co-cultivation for 1, 2, 3, 4, and 5 days at co-cultivation temperature of 24°C, 28°C, 32°C or 36°C, the fungal-bacterial cells on the filter paper were transferred to PDA amended with hygromycin B and cefotaxime to eliminate the *A. tumefaciens* cells. Individual transformants were transferred after 4 to 6 days to PDA amended with hygromycin B. In all experiments, *C. capsici* conidiospores co-cultivated with uninoculated induction medium were included as a negative control. All experiments were replicated and results were analysed using ANOVA.

Analysis of transformation events. The frequency and randomness of T-DNA integration in the fungal genome was determined by PCR and Southern blot.

RESULTS AND DISCUSSION

Agrobacterium was successfully used to transform *C. capsici* conidiospores and mycelium (Figure 1). The biological differences among fungal species can influence transformation efficiencies in different filamentous fungi (2). Following optimisation, high transformation efficiencies were routinely obtained for *C. capsici*.

Conidiospore density. Transformation efficiency was consistently found to be optimum at the conidiospores density of 10^6 and 10^8 conidiospores per ml. Hence, subsequent transformations were carried out using 10^6 conidiospores per ml.

Ratio of fungal spores to bacterial cells. The highest transformation efficiency was obtained with equal volume of the mixture.

***Agrobacterium* strains.** *A. tumefaciens* AGL1 strain produced more transformants (16.2% more) than LBA4404 regardless of the binary vector used.

Plasmid type. pJF1 and pPK2 plasmids provided similar transformation efficiencies. In contrast, pKHt plasmid produced significantly less transformants ($p < 0.01$).

Acetosyringone. Transformants were only obtained when the medium was supplemented with the wound respond molecule, acetosyringone.

Co-cultivation time. The number of transformants increased depending on the increased period of co-cultivation. Prolonged co-cultivation period however, brought about excessive growth of the fungus and resulted in difficulty in isolating single colonies.

Co-cultivation temperature. The optimal co-cultivation temperature for *C. capsici* transformation was 24°C. The number of transformants dramatically decreased when the co-cultivation temperature was increased to 28°C and at 32°C no fungal colonies were observed. Lower temperatures appeared to be beneficial towards the T-DNA transfer (2).

PCR and Southern blot analysis. PCR of transformants detected the T-DNA insertions. Southern analysis indicated that 87.5% of the transformants had a single copy of the T-DNA integrated randomly in the fungal genomes.

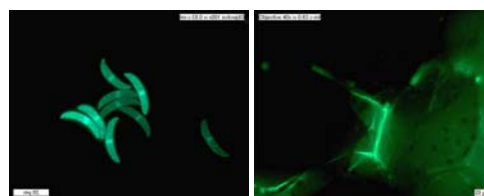


Figure 1. Expression of *GFP* in conidiospores and in hyphae colonising, intramurally, infected parenchyma cells of chilli pepper, as visualised with green fluorescent filter.

Based on an optimised protocol, *Agrobacterium* transformation approach has proven to be an efficient method in transforming *C. capsici*.

ACKNOWLEDGEMENTS

We thank Professor L. Vaillancourt (University of Kentucky), Professor S. Covert (University of Georgia) and Professor S. Kang (The Pennsylvania State University) for supply of pJF1, pPK2 and pKHt plasmids, respectively.

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60 Infection process of endophytic *Colletotrichum gloeosporioides* on cacao leaves

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INTRODUCTION

Colletotrichum species are commonly isolated as endophytes from leaves and fruits of tropical plants. In preliminary surveys *Colletotrichum* spp. accounted for 29–48% of isolates sampled as endophytes from leaves of the cacao tree (*Theobroma cacao*) in four sites in Australia and Papua New Guinea. By definition endophytes do not cause disease symptoms at the time they are isolated from plant tissue. The infection process and subsequent tissue colonisation has been elucidated for only a few endophyte host interactions, none of which include tropical plants.

Colletotrichum species can penetrate plant tissue through wounds, natural openings such as stomata or by penetration of the plant cuticle¹. They are often categorised into three groups: intracellular hemibiotrophs, subcuticular intramural colonisers and those that display a combination of the two infection strategies¹. Intracellular hemibiotrophs first grow biotrophically in host tissue before switching to a necrotrophic stage which results in symptom development. Subcuticular intramural pathogens grow beneath the cuticle and cause dissolution of the epidermal cell walls. The aim of this research was to investigate the infection process of an endophytic isolate of *C. gloeosporioides* on *T. cacao* leaves.

MATERIALS AND METHODS

An isolate of *C. gloeosporioides* was isolated from apparently healthy leaf tissue of *T. cacao* in Far North Queensland, Australia. Young and mature leaves of cacao were sprayed with a 1×10^5 conidia/mL suspension to runoff. Leaf tissue was sampled for observations every 2h for 16h, at 24h and then every 24h for 6 days. Tissue was cleared for 4h at 60°C followed by 20h at room temperature in a solution of 0.15% trichloroacetic acid in 3:1 ethanol:chloroform. Tissue was immersed in 0.025% aniline blue in lactoglycerol for 1h at 60°C followed by 23h at room temperature in order to stain fungal hyphae. Experiments were repeated at least three times.

RESULTS

Conidia began germinating within 6 hours post inoculation (hpi), usually giving rise to one and rarely two germ tubes. Appressoria were produced at 8–10 hpi, either directly or at the end of a short germ tube and became melanised by 12 hpi. Infection pegs were produced predominantly over cell walls at 12–16 hpi in both young and mature leaves. Stomatal penetration was never observed. Infection vesicles were visible at 3 days post inoculation (dpi) in young leaves and appeared as thick, highly lobed hyphae which filled the epidermal cell directly beneath the infection peg. At 4–5 dpi infection vesicles had branched into narrow secondary hyphae which penetrated cell walls and grew inter- and intra-cellularly in young leaves (Fig. 1). Infection vesicles formed in mature leaves 4–5 dpi and had a similar appearance to those in young leaves (Fig 2). In mature leaves, infection was restricted to the initial cell in which the infection vesicle formed over the 6 days of observation.

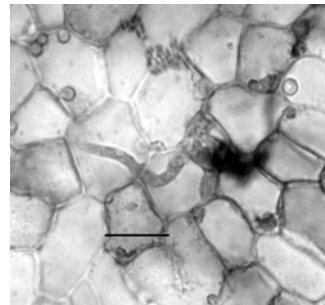


Figure 1. Primary hyphae of *C. gloeosporioides* colonising epidermal cells of *T. cacao* leaves 4 dpi. Bar = 10µm

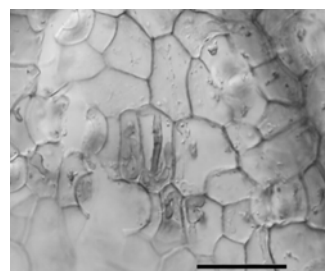


Figure 2. Primary hyphae of *C. gloeosporioides* restricted to the epidermal cell directly below appressoria (5 dpi). Bar = 20µm).

DISCUSSION

Endophytic *C. gloeosporioides* on *T. cacao* leaves can be categorised as an intercellular hemibiotroph. Infection was observed in epidermal cells directly beneath the appressorium and no subcuticular intramural growth was observed. The infection process did not differ on young and mature *T. cacao* leaves in the first 3 dpi. Following this, colonisation was more rapid in young leaves and led to the production of disease symptoms. Infection in mature leaves remained biotrophic and fungal growth appeared to cease after infection and colonisation of one epidermal cell. The length of the biotrophic, asymptomatic phase has been correlated the redox state² and pH of the host tissue³ in other *Colletotrichum*-host interactions. Factors affecting the infection process in *T. cacao* are currently being investigated.

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2 A *Phytophthora* sp. is the cause of jackfruit decline in the philippines

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INTRODUCTION

Jackfruit is a very popular domestic fruit in the Philippines. It has a wide distribution and is cultivated throughout the country. A survey of jackfruit growers in the Eastern Visayas indicated that wilt disease was the main constraint to improved productivity. In some areas up to 90% of jackfruit trees are affected by wilt disease, manifested by leaf yellowing, defoliation, girdling stem lesions and rot. Previous attempts to identify the pathogen yielded a range of fungal, nematode or bacterial isolates, none of which proved pathogenic. Accurate identification of the cause of the decline syndrome is imperative for the control of the disease. This study seeks to isolate and identify the pathogen causing jackfruit wilt and to evaluate a range of disease management strategies through participatory action research.

MATERIALS AND METHODS

Affected roots, stem canker lesions and soil from near infected trees was suspended in water and baited with flower petals¹. Lesions that developed within 2 days were surface sterilised and plated on Potato Dextrose Agar, Carrot Agar and Onion Agar, supplemented with benomyl, nystatin and streptomycin. Pure cultures were re-introduced to flower baits to induce sporangia, zoospore and chlamydo-spore formation for inoculation of detached jackfruit leaves and seedlings.

RESULTS

Wilt disease was recorded in all the fields examined within Leyte and Samar islands, at an incidence of 5–90% of trees. Areas with very high incidence were typically subject to periodic heavy flooding, particularly during the rainy season. Yield losses were estimated to be range from 5–80%. Field visits and farmer interviews showed that almost all of the farmers were unaware of the cause of the disease or appropriate management strategies.

A *Phytophthora* species was consistently isolated from affected jackfruit roots and canker lesions, and from soil collected near infected plants. Pathogenicity was confirmed when the isolates produced typical wilting symptoms on inoculated plants (Fig. 1a) and leaf lesions (Fig.1b).



Figure 1. a. Un-inoculated (leftmost) and inoculated jackfruit seedlings showing different degrees of wilting. b. The isolated pathogen causes leaf lesions.

In pure culture the mycelium of the pathogen is white with a stellate growth pattern. Sporangia are seldom produced in PDA, Carrot or Onion Agar, but readily produced when pure cultures were re-introduced to flower petal baits. The pathogen produced spherical to ovoid sporangia with an average length of 41.5 μm and breadth of 26.5 μm . Sporangia have a relatively long pedicels, are semi-papillate to papillate and release

zoospores through a vesicle before they separate and swim away (Figs 2a and 2b). The isolated *Phytophthora* species produces abundant intercalary and terminal chlamydo-spores when re-introduced to flower baits.

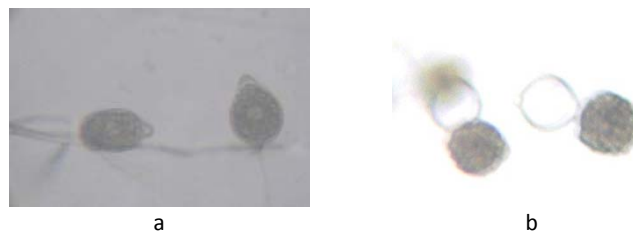


Figure 2. a) *Phytophthora* sporangia, b) zoospores exiting via spherical vesicles.

Participatory action research (PAR) disease management trials are being established by researchers, extension officers and jackfruit farmers in Leyte and Samar Islands. Nine PAR trials have been established in Leyte and Samar to test a range of management options including field sanitation, organic amendments, improved drainage, good nursery practices and chemical control in managing wilt disease.

The identification of the pathogen associated with the symptoms of decline and wilt will enable the development of more effective, targeted management strategies.

ACKNOWLEDGMENTS

This research is funded by ACIAR HORT/2006/067/2

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3 The effects of calcium chloride and calcium carbonate on germination and growth of *Colletotrichum acutatum* and *Penicillium expansum*

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INTRODUCTION

Calcium chloride (CaCl₂) and calcium carbonate (CaCO₃) have been found to enhance the biocontrol activity of yeasts against a range of diseases. A number of factors including inhibition of the pathogen by the compounds may account for this (1). The aim of this research was to investigate whether these compounds inhibited germination and growth of *Penicillium expansum*, the causal agent of blue mould of apples, and of *Colletotrichum acutatum*, which causes bitter rot of pome fruit (2,3).

MATERIALS AND METHODS

Germination (%) and germ-tube length (µm) were assessed after 20–24 h at 20°C for three *C. acutatum* isolates made up in 0, 10, or 20 mg/ml CaCl₂. *P. expansum* requires exogenous nutrients to germinate well, thus germination assays were conducted in 0, 12.5 and 25% apple broth with 20 mg/ml CaCl₂ for three *P. expansum* isolates. For each combination, the per cent germination of 150 conidia and germ-tube length of 30 conidia was recorded.

Because suspended CaCO₃ made it difficult to observe conidia in germination tests, the effects of CaCO₃ and CaCl₂ (each 20 mg/ml) on the three isolates of *P. expansum* and two of *C. acutatum* were also investigated by dilution plating on 0, 12.5 and 25% apple broth agar with and without CaCl₂ and counting colony forming units (cfu) after 4–7 days.

All experiments were conducted twice and data were analysed using analysis of variance. Germ-tube lengths were log₁₀ transformed and colony counts were square-root transformed before analysis. *P*=0.05 was used to assess significance.

RESULTS AND DISCUSSION

Increasing concentrations of CaCl₂ increased the germination and germ-tube length of *C. acutatum*, although there was a significant interaction between the factors studied (Table 1).

Table 1. Mean per cent germination and germ-tube length (log₁₀ transformed) for each *Colletotrichum acutatum* isolate and CaCl₂ concentration.

Isolates	Germination (%)			Germ-tube length (µm log ₁₀)		
	CaCl ₂ (mg/ml)			CaCl ₂ (mg/ml)		
	0	10	20	0	10	20
C4	57	57	85	0.56	0.45	0.34
C7	41	69	100	0.39	0.67	0.70
C8	71	51	85	0.45	0.27	0.70
	s.e.d. interaction 17.2			s.e.d. concentration 0.097		

P. expansum conidia did not germinate in water alone. In apple broth, the addition of CaCl₂ significantly reduced mean germination of *P. expansum* from 37 to 16% and germ-tube length from 0.382 to 0.160 compared with the control. There was no difference in germination and germ-tube length between the two concentrations of apple broth.

On apple broth agar, no differences between treatments were observed and therefore results are given only for agar with no apple broth.

For all *P. expansum* isolates, the addition of CaCO₃ to the agar resulted in significantly fewer cfu than in the CaCl₂ and water only treatments. The addition of calcium carbonate did not significantly affect cfu counts of *C. acutatum* isolates.

In conclusion, neither CaCl₂ nor CaCO₃ reduced germination, germ-tube growth or cfu counts for *C. acutatum*. This suggests that some other mode of action contributes to enhancement of bitter rot control when these compounds are combined with yeasts. In contrast, the germination, germ-tube growth and cfu counts for *P. expansum* were all reduced by the addition of CaCl₂ and CaCO₃ to the growth medium suggesting that this direct inhibition could contribute to the improved blue mould control in apples when yeasts are combined with these compounds.

ACKNOWLEDGEMENTS

Thanks to the New Zealand Foundation for Research, Science and Technology for funding this project (C06X0302).

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45 Fungal endophytes of the Boab species *Adansonia gregorii* and other native tree species

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INTRODUCTION

In southern Africa dying Boabs have been reported and subsequent surveying of these trees has indicated the presence of the fungal pathogen *Lasiodiplodia* which may be the cause of the decline. Due to the close genetic relationship of the African and Australian Boabs and the fact that these two continents share a large amount of floral families, they may subsequently also share the pathogens of many of these plants. The surveying of the otherwise healthy Australian Boab and surrounding tree species deemed a prudent course of action.

In this study Boabs were surveyed in 25 sites in the Kimberley region and material was also taken from surrounding tree species at 3 sites. Endophytic fungi that were isolated from these samples were identified using both molecular and morphological data and seven new species were described (2). The pathogenicity of identified species to Boabs was determined. This is the first study to identify endophytes of the *Adansonia* and to conduct pathogenicity trials on these trees.

MATERIALS AND METHODS

Stem and leaf material was collected from a range of sites across the Kimberlys, Western Australia. Material was taken from *Adansonia gregorii* and a range of native flora in the same area. Endophytes were isolated using standard protocols (1).

Lesion development in seedling tap roots. 24 isolates that represented the genetic diversity of samples collected were used to inoculate the tap root of four-month-old Boab seedlings. They were inoculated by using a sterile scalpel blade to make a small lateral incision along the middle of the carrot. Into which a 1 cm² agar plug colonised with mycelium was inserted face up. This was then lightly wrapped with parafilm. There were 24 isolates plus controls (10 replicates of each). Tap roots from each replicate were placed in random order onto wooden racks inside plastic. The containers were then sealed with aluminium foil and tape and placed into a 25C room and left for 4–5 days. After four days lesion development in the tap roots were measured. The lesions presented as a rotted mass that could easily be scraped out of the tap root. The inoculated tap root was weighed, the lesion was scraped out and the carrot was re-weighed immediately. The lesion length and width was also measured.

Lesion development in young trees. 2–3 year old Boab trees were harvested in Kununurra from commercial Boab growers "Boabs in the Kimberlies.". They were planted within 2 weeks of initial removal into one meter long PVC pipes in a potting medium of 1/3 coarse river sand and 2/3 potting mix and were watered twice a day for ten minutes by an automatic dripping system. Nine isolates were selected from the tap root trial. Boab stems were inoculated in the same manner as the roots. There were 5 replicates for each of the 9 isolates and also non-inoculated controls. After 6 months trees were assessed for leaf cover and stems with lesions were harvested. The width, length and depth of lesions were measured using callipers and a ruler. The stems were cut in half at the centre of the initial mycelium plug insertion in order to determine the depth of lesion development.

At the extreme margin of the lesions the wood was cut away using a knife in order to establish the extent of interior lesion development.

RESULTS AND DISCUSSION

433 fungal isolations were made, 282 of these consisted of isolates belonging to Botryosphaeriaceae including species of *Neofusicoccum*, *Pseudofusicoccum*, *Lasiodiplodia* *Dothiorella* and *Neoscytalidium*.

For the trial with tap roots, isolates from the *Lasiodiplodia theobromae* complex produced the largest lesions. *Neofusicoccum ribis* and *Neoscytalidium novaehollandia* caused moderate lesions and isolates of *Lasiodiplodia crassispora*, *Dothiorella longicollis*, *Pseudofusicoccum adansoniae* and *Fusicoccum ramosum* all caused minor lesions indicating low virulence.

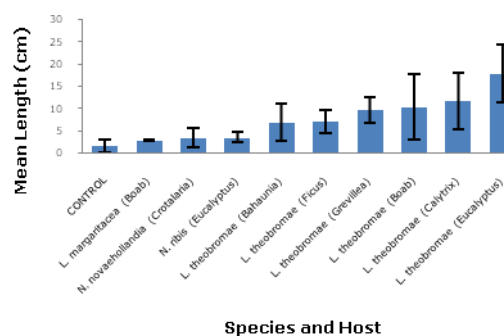


Figure 1. Mean length(cm) of lesions in 2–3 year old boab trees. Standard deviations are represented by the error bars.

The results of the tree trial (Figure 1) confirm the results of the preliminary investigation, *L. theobromae* was found to be significantly more pathogenic than other species considered in the study. The lesions produced from inoculation of boab stems by *L. theobromae* resulted in the lesions lengths ranging from 3 cm to 25cm (mean= 10.68cm). *N. ribis* and *Neoscytalidium novaehollandia* both exhibited similar lesion severity (means= 3.46 cm and 3.54 cm respectively).

This trial indicates the potential threat that *L. theobromae* presents to the iconic Boab trees. Recently a dying Boab in Broome was reported with similar disease symptoms those of dying Boabs in South Africa and similarly, *L. theobromae* was the only pathogen isolated from the cankers. As shown in this trial, endophytes such as *L. theobromae* are capable of causing disease and killing Boabs in Australia.

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61 An emerging nematode pest on bananas?

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INTRODUCTION

The focus of nematode control on bananas in Australia and worldwide has been on *Radopholus similis* (burrowing nematode) as the key nematode pest. International research and breeding programs have been active in managing and selecting cultivars for resistance to this nematode. However, recent surveys of African bananas have found *Pratylenchus goodeyi*, *P. coffeyi*, *Meloidogyne* spp. and *Helicotylenchus multicinctus* as key nematode species.

P. goodeyi (lesion nematodes) is considered to be indigenous to Africa where its distribution has been limited to the cooler highland growing areas including Central, Eastern and West Africa. It is considered that *P. goodeyi* has the potential to become an important pest of bananas where they are grown in cooler climatic zones of the Mediterranean and Middle Eastern countries. Bananas, as well as a number of other tropical crops, have been planted in the subtropical regions of South America, southern Africa, the Mediterranean basin, Australia and southern China. *P. goodeyi* has been recorded in the Canary Island, Crete and Egypt and Australia (1). However, it has recently also been found in warmer banana production areas of Africa (2).

P. goodeyi can invade and feed in the root and corm tissue of banana plants. It causes similar symptoms and destruction as caused by *R. similis* including root lesions, stunted growth, reduced bunch weight and toppling of the bunching pseudostem.

There is concern that the distribution of *P. goodeyi* may spread throughout subtropical banana production areas and it may also move into warmer, tropical production areas in Australia, following the recent experience in Africa.

MATERIALS AND METHODS

Root samples were submitted from several banana growing properties from northern NSW for the extraction of *R. similis* for molecular analysis. Roots were sliced open lengthwise and placed in a misting chamber for 5 days for the extraction of nematodes (3). Nematodes were caught by passing the washing from the mister over a 38 µm sieve. The washings were then examined under a compound microscope for the presence of nematodes and positive identifications made. For this accurate identification, nematodes were picked from solution using an eyelash and placed under higher magnification to further distinguish between *R. similis* and *P. goodeyi*.

Single mature female nematodes of both *R. similis* and *P. goodeyi* were placed on a sterile carrot (*Daucus carota*) disc in order to initiate a single genotype isolate (4) for further experimental work.

RESULTS AND DISCUSSION

The root samples from these farms were found with a high incidence of *P. goodeyi*. Furthermore, some farms had exclusively *P. goodeyi*, and not *R. similis* as was believed. This suggested that *P. goodeyi* may be increasing in numbers and importance within banana plantations of NSW and south-east Queensland.

As *P. goodeyi* was thought to have originated from the African highlands it is still unclear how this nematode arrived in Australia. However, with the number of sites with *P. goodeyi* increasing in NSW and the experience in Africa of *P. goodeyi* moving into the hotter production areas, the Australian banana industry needs to be aware of the distribution of these nematodes to avoid a repeat of the African experience.

The movement of infested planting material and soil could increase the spread of *P. goodeyi* to additional banana growing areas within Australia, therefore, growers need to adhere to the clean planting material policy and be aware of the possibility of spread with infested soil and machinery.

Accurate identification of the nematode species is essential to develop management options such as crop rotation and use of resistant cultivars. Surveys need to be undertaken to establish the spread of this nematode, as well as resistance screening of rotation crops and of new banana cultivars.

ACKNOWLEDGEMENTS

Funding for this work was provided by Queensland Primary Industries and Fisheries.

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4 A sensitive PCR test for detecting the potato cyst nematode (*Globodera rostochiensis*) in large volume soil samples

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INTRODUCTION

Potato cyst nematode (PCN) *Globodera rostochiensis*, a devastating plant pathogen worldwide, impacts potato production and affects market access. PCN was detected between 1986 and 1989 on six properties (a total of 15ha) in the metropolitan area of Perth, Western Australia (WA). A strict quarantine and eradication program was immediately implemented, and no PCN has been detected anywhere in the state since. With almost 20 years since the last detection, WA is now in an excellent position to re-claim Area Freedom from PCN.

We are developing a sensitive PCR test to enable presence/absence of PCN to be determined directly from large soil samples for confirmation of Area Freedom for the state. PCR offers an alternative to traditional microscopic detection of PCN, which is time-consuming and prone to operator error, particularly if cysts are present in low numbers.

MATERIALS AND METHODS

Field survey. Proving a 'negative' is always a challenge. With this in mind, survey methods were tailored to generate data to show with the highest possible confidence that PCN no longer occurs in WA. At all survey sites, 50g soil samples to a depth of 15 cm were collected on a 5 x 5 m grid pattern across entire fields. This resulted in collection of approx. 20 kg/ha, all of which was processed (without sub-sampling) by the Fenwick method for total organic matter extraction. This sampling regime is far more intensive than any standard worldwide. All organic matter samples will be assessed using the PCR test under development.

PCR test. There are numerous technical challenges when amplifying DNA extracted directly from soil (e.g. incomplete cyst/egg lysis, DNA adsorption to soil, co-purification of PCR inhibitors, and degradation of target DNA). To reduce inputs, it is necessary to develop methods that maximise sample area per test without compromising assay integrity. PCR analysis of soil has usually been done with samples of only 1 to 15g. In contrast, we are developing a novel strategy to test 20kg pooled soil samples (each representing assessment of 1ha sampled on a 5 x 5m grid) for presence/absence of PCN.

Due to quarantine against the use of PCN, we are developing methodologies using Cereal Cyst Nematode, *Heterodera avenae* (CCN). The goal is to develop the technology for routine detection of 10 cysts in a 20kg soil sample. Once optimised, detection methodologies will be validated in blind studies using PCN-infested soil in New Zealand.

RESULTS AND DISCUSSION

Results are encouraging, with as little as 1 CCN cyst detected in 20kg of soil (Fig. 1). Since the average PCN cyst contains approx. 400 eggs, this is equivalent to detection of approx. 0.02 eggs/g soil which is a detection level that could identify extremely low levels of infestation.

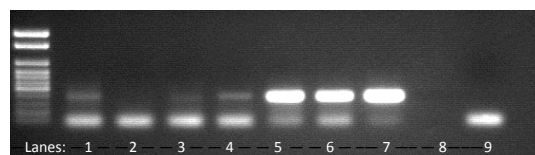


Figure 1. PCR results from samples with 1-200 CCN cysts. Lane 1: 1 cyst; Lane 2: 5 cysts; Lane 3: 10 cysts; Lane 4: 20 cysts; Lane 5, 50 cysts; Lane 6: 100 cysts; Lane 7: 200 cysts; Lane 8: blank; Lane 9: H₂O.

Although we have been able to detect only 1 CCN cyst in 20kg of soil, reliability of the test is more consistent for 10 cysts/20kg of soil (Fig. 2). This represents detection of approx. 0.2 eggs/g soil, which is far below national and international standards.

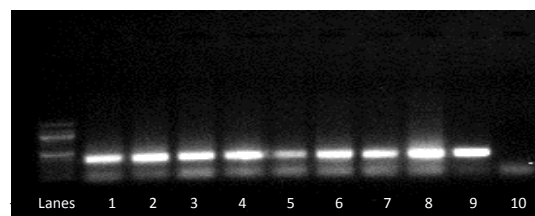


Figure 2. PCR results using target DNA extracted from samples with 10 CCN cysts. Lanes 1-4: 5uL target DNA; Lanes 5-8: 10uL target DNA; Lane 9: +ve control; Lane 10: H₂O.

Currently, the test is being refined to eliminate the effects of contaminants and inhibitors in the soil. Ways to increase reliability of the PCR test in different soil types are also being assessed. For example (Fig. 3), results from a pilot trial have shown that detection of CCN DNA from Albany soil (Humic Podzols) was more reliable than from Busselton soil (Jindong Sandy Loam).

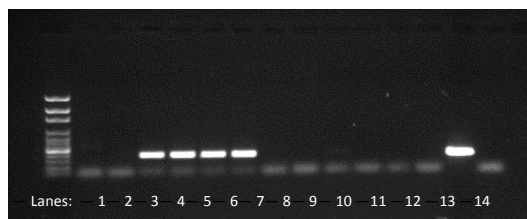


Figure 3. PCR results using 5ul of target DNA extracted from 10 CCN cysts in two different soil types (Albany and Busselton). Lanes 1 and 2: -ve control for Albany soil; Lanes 3-6: Albany soil; Lanes 7 and 8: -ve control for Busselton soil; Lanes 9-12: Busselton soil; Lane 13: +ve control; Lane 14: H₂O.

Once optimised, this test could have potential application for detection of other pests and pathogens that can be found in soil organic matter.

ACKNOWLEDGEMENTS

Horticulture Australia Ltd and the Potato Growers' Association WA provide funding. WA growers allowed us to sample fields. Larry Hegarty (Potato Marketing Corporation) supplied planting, harvest data and maps. Dyane Jardine and Ali Bhatti provided field and lab technical support. Peter Philippe (WA Quarantine Inspection Service) allowed access to historical PCN sampling data.

62 *Phellinus noxius*: brown root rot is increasing in importance in the Australian avocado industry

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INTRODUCTION

Phellinus noxius is an indigenous wood decay basidiomycete, common in tropical and subtropical rainforests of eastern Australia, Asia, the Pacific, central America and Africa. *Phellinus noxius* causes root and lower stem rot ("brown root rot") disease of native and introduced trees planted on former rainforest sites. Overseas, it has caused significant losses through tree deaths in hosts such as rubber, mahogany, teak, cocoa, longan, litchi, pear, persimmon and *Acacia mangium* (widespread plantings in south east Asia for pulpwood).

Infection takes place when roots contact infested woody matter present in the soil, and thus spread is most likely via root-root contact. Trees can suffer a rapid decline, and foliage may transform from green and healthy to wilted and dead within a few weeks (Plate 1). Decline in older trees can be more gradual, with some mature infected trees surviving for many years. One key diagnostic feature is the presence of a thick brown mycelial mat with a white actively growing margin that melanises with age, which can be found growing on the root and stem surfaces (Plate 2). Fruiting bodies, although uncommon, occur in two forms. The resupinate form is seen on the underside of fallen logs, and between buttress roots of *Ficus* spp., and the bracket form is more often seen on dead trees in higher rainfall areas such as northern Queensland.

The disease leads to significant losses in hoop pine plantations in Queensland, and in broadleaf hosts (eg. *Ficus* spp.) in urban parks and gardens (2). Death of avocado trees successively down rows was first noted on the Atherton Tablelands in QLD in 2001. The first positive identification of *P. noxius* causing tree death in avocado occurred in 2002 from the Maleny district of the Sunshine Coast hinterland in Queensland.

This paper reports on a scoping study undertaken to assess the spread and severity of brown root rot in major avocado growing areas of north eastern Australia.



Plate 1. Quick decline of an avocado tree, laden with fruit.

MATERIALS AND METHODS

Several orchards across the Atherton Tablelands and Childers/Bundaberg production regions, representing areas of approximately 650 and 300 km², respectively, were visited. Selected additional properties in other areas of SE QLD or northern NSW were also visited. Samples from actively growing infection stockings were collected and plated onto malt extract agar containing 1%w/v streptomycin and 1ppm benomyl. Cultures were identified by morphological features of the hyphae.

RESULTS

P. noxius was confirmed on 17 out of 18 properties visited on the Atherton Tablelands, including in mango at 2 sites. It was also confirmed from 3 (and suspected on a further 2) orchards in the Childers/Bundaberg area, where 2 properties visited were apparently free of the disease. It has been confirmed on one orchard at Maleny and 2 orchards in northern NSW. Losses were particularly severe (approx. 10% tree death in affected blocks) in at least 4 orchards visited, and attempts to replant in infested soil failed. To date, no fruiting bodies have been found on avocado.



Plate 2. The characteristic infection "stocking" at the base of avocado trunk

DISCUSSION

Effective management relies on complete removal of dead and dying trees and their roots, and one apparently healthy tree on either side. Root barriers can then be installed to prevent roots from uninfected trees coming into contact with infected debris in soil. There is currently no effective chemical control.

ACKNOWLEDGEMENTS

We acknowledge M. Weinert (QPIF, Mareeba) and E. Dunn (Crop Tech, Bundaberg), for assistance in organising the orchard visits and numerous avocado growers for their cooperation and access. The project is funded by Avocados Australia Ltd. and HAL.

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5 Management strategies to economically control blackspot and maximise yield in new improved field pea cultivars

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INTRODUCTION

Field pea production in South Australia has remained constant at approximately 120,000 ha since the mid 1990s, although plantings have increased in medium to low rainfall areas. Blackspot, caused by a complex of fungi i.e. *Mycosphaerella pinodes*, *Phoma medicaginis* var. *pinodella*, *Ascochyta pisi* and *Phoma koolunga* (1), is the most common disease in field peas. Research on blackspot in the 1990s, based in traditional areas and on traditional late-maturing trailing type peas i.e. cv. Alma, found that foliar fungicides were uneconomic but delaying sowing minimised blackspot infection from airborne spores. Delayed sowing is still a major recommendation for the pea industry across South Australia (2). Given the expansion into low rainfall areas and increasing frequency of low rainfall seasons, the potential yield loss through delayed sowing is often now greater than the loss from blackspot. Furthermore, fungicide costs have reduced and this practice may now be economic in some environments. The pea industry has also adopted higher yielding cultivars including early maturing erect semi-leafless types i.e. cv. Kasper. Agronomic trials were conducted in 2007 and 2008 to identify economic strategies to control blackspot in new improved pea cultivars, and to identify optimum sowing dates in low to medium rainfall areas for these cultivars.

MATERIALS AND METHODS

Trials were sown at three sites each season, viz. high rainfall (450mm per annum) at Kingsford in 2007 and Turretfield in 2008; medium rainfall (400mm per annum) at Hart in both seasons; low rainfall (325 mm per annum) at Minnipa in both seasons. The high and medium rainfall sites had three sowing times and the low rainfall site had two sowing times. First sowing occurred within a week of the break of the season (first week of May) at each site and subsequent sowing times were at intervals of three weeks. Trials were split plot design, with time of sowing as the main block, with three replicates. Cultivars included the conventional trailing types Alma and cv. Parafield (the latter at Minnipa only), and the new erect semi-leafless types including the current commercial cultivar Kasper and advanced breeding lines WAPEA2211 and OZP0602 (the latter in 2008 only). Fungicide treatments were the seed treatment P-Pickel T[®] (thiram plus thiabendazole, 200 ml/100kg seed), a foliar application of mancozeb (2 kg/ha) at 9 node growth stage, foliar applications of mancozeb at 9 nodes plus early flowering growth stage, P-Pickel T[®] seed dressing plus a foliar application of mancozeb at 9 nodes, foliar applications of chlorothalonil (2 L/ha) every fortnight (i.e. disease control) and an untreated control. Disease was assessed regularly, 2 or 3 weeks apart, throughout the growing season, and recorded as % leaf area diseased (%LAD) in the early stages of the epidemic, or as % of nodes infected (%ND) in the later stages of the epidemic. Plot yields were recorded as tonnes per hectare. Significant differences identified by analyses of variance were separated on $P < 0.05$.

RESULTS

Disease severity reached between 25% and 60%ND in medium and high rainfall trials but lack of rainfall during spring stopped further disease progress in both seasons. Low rainfall trials

developed less than 5% LAD due to dry conditions. Delayed sowing reduced disease levels by 30% less than the untreated controls throughout the season. P-Pickel T[®] reduced disease levels by 50% for 6–8 weeks after sowing but no differences were detected by the end of the season. Foliar fungicides resulted in a small (6–12%) reduction in disease severity, but this did not translate into additional grain yield due to the dry spring. Infection appeared earlier in Alma and disease remained at higher levels than in the other varieties. Kasper had higher disease levels than WAPEA2211 which in turn had higher levels than OZP0602.

Yield in early sown plots was significantly higher than later sown plots in 2007, but not in 2008 due to erratic high temperatures and frosts in spring. However a variety interaction occurred. In 2008 the early flowering line, OZP0602, was the highest yielding line when sown at the mid sowing time, and it had similar highest yield to Kasper at the early sowing time. Kasper yield decreased with later sowing. The older conventional variety Alma was the lowest yielding variety and showed a variable response to sowing date.

DISCUSSION

Blackspot was reduced in new cultivars and breeding lines, indicating that improved blackspot resistance is becoming available to the Australian pea industry. The semi-leafless more erect pea types are better adapted to earlier sowing dates than the older conventional types due to a combination of reduced biomass, more erect plant type and slower disease development in the early growth stages. Maximum grain yields of Kasper were achieved by sowing early but this exposes Kasper to higher disease and frost risks. The breeding line, OZP0602, was equally as high yielding in first and second time of sowing. Consequently, this line does not need to be sown early to maximise yields, providing a safer option when sowing needs to be delayed. Foliar fungicides slightly reduced disease, but this did not translate into yield gains in these trials due to the dry springs. Anecdotal evidence, from commercial crops grown in average rainfall seasons, has shown that similar small reductions in disease lead to economical yield gains. Further research is required to confirm this in trials with more favourable spring rainfall.

ACKNOWLEDGEMENTS

This research was funded by South Australian Grains Industry Trust.

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63 Dispersal potential of *Gibberella zeae* ascospores

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INTRODUCTION

Fusarium head blight (FHB) of wheat, caused by the fungus *Gibberella zeae* (anamorph *Fusarium graminearum*) is a disease that occurs sporadically in the Liverpool Plains region of Northern NSW. The fungus is also a pathogen of maize, causing Gibberella stalk and ear rots, and an asymptomatic endophyte of sorghum. The pathogen survives in the residues of these hosts, and in spring and autumn, perithecia form on these residues and forcibly discharge ascospores into the air (1).

The potential for long distance dispersal of these ascospores has been examined in North America (1, 2, 3), where spores have been recovered at least 3km from the nearest inoculum source and at 60m above the earth's surface (2). This suggests that where there is a significant regional source of inoculum, localised control of infested residue through rotation or tillage practices may not effectively reduce the risk of FHB in individual fields (1).

While inoculum levels and potential for dispersal are traditionally greater in North America compared to Australia, due to more favourable climatic conditions and the greater presence of maize within the farming system, evidence to support longer distance dispersal has been observed in the Liverpool Plains during 2005, when wheat crops free of inoculum had moderate levels of FHB.

To determine the potential for long distance dispersal of ascospores under Australian conditions, a spore trapping experiment was established during October, 2008.

MATERIALS AND METHODS

A centre pivot irrigation field (80ha) in Spring Ridge (latitude 31° 31'2.1"S longitude 150°14'6.3"E) was identified as a source of inoculum due to significant amounts of *G. zeae* perithecia on 6 month old maize residue and high levels of FHB and perithecia on a cv. *Beaufort* wheat crop.

Spore traps, standing 1m in height were placed at 50m intervals in a north easterly direction into a field 12 months fallow from Chickpeas, to a distance of 250m from the inoculum. Traps were also placed at 50m intervals into the wheat crop to a distance of 250m into the crop. Traps consisted of four 90mm petri dishes containing *Fusarium*-selective medium with increased rates of antibiotics, exposed to the atmosphere from sunset to sunrise the following morning. Exposure of the plates was timed to follow an irrigation event to the wheat crop of equivalent to 15mm of rainfall 24 hours prior.

Plates were recovered and incubated for 3 days under alternating light and dark conditions with temperatures at 24°C and 22°C respectively. A random subset of the colonises were subcultured from each plate and identified morphologically. Spore counts were taken from each plate and used to determine the number of *G. zeae* ascospores intercepted.

RESULTS

Ascospores of *G. zeae* were recovered at all locations and ranged from 90 cfu per plate at 250m from the inoculum source to 750 cfu per plate within the wheat crop. The pattern of dispersal of

spores away from the source of inoculum closely fitted an exponential curve ($R^2=0.97$) (Figure 1).

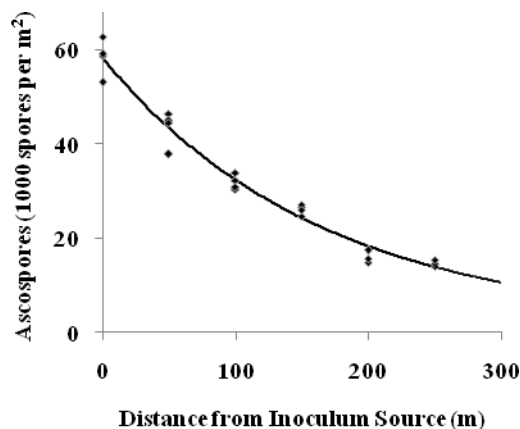


Figure 1. *G. zeae* ascospore deposition away from inoculum source. The pattern of deposition closely follows the exponential curve $y = 1.52 + 56.99 \times 0.99^x$ $R^2 = 0.97$

DISCUSSION

The pattern of ascospore dispersal agrees with previous reports of the incidence of disease away from an inoculum source being described by an exponential model (3). The results also suggest that recovery of spores at distances greater than 250m is likely. Extrapolation of the model to 500m suggests that 4000 spores/m² would be deposited nightly. Whether this level of deposition is sufficient to initiate disease however is yet to be established.

This experiment demonstrated that spore release events can be triggered by overhead irrigation events. The timing of irrigation events on wheat crops following maize should attempt to avoid irrigating during anthesis, at which wheat is susceptible to infection. Residue management may also be necessary to reduce the risk of FHB in such situations.

ACKNOWLEDGEMENTS

The research was completed with the assistance of a GRDC Grains Research Scholarship.

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25 Effect of avocado crop load on postharvest anthracnose and stem end rot, and cations and phenolic acid levels in peel

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INTRODUCTION

Crop load (or tree yield), rootstock and mineral nutrition in the flesh can influence quality of 'Hass' avocado fruit in terms of anthracnose and internal flesh disorders (1,2). While fruit from higher yielding trees are often smaller, they have been reported to have less anthracnose (*Colletotrichum gloeosporioides*) and higher Ca (2). Ca is thought to strengthen cell walls making them more resistant to fungal pectolytic enzymes. The balance between N and Ca is also critical as excessive nitrogenous fertiliser can result in increased photosynthetic activity in leaves, outcompeting the developing fruit for water and Ca.

Phenolic compounds are abundant in plants and have important roles as/in cellular support materials, eg. lignins, detoxification, components of flower and fruit colour (eg. anthocyanins), protection against herbivore predators, signal molecules, and as phytoalexins. Thus, they contribute to disease resistance mechanisms of plants.

We investigated the effect of crop load on anthracnose and stem end rot (caused primarily by *Botryosphaeria* spp. but also *C. gloeosporioides*) postharvest diseases in 'Hass' avocado in two field seasons, and measured cation concentrations (particularly Ca and N) and total soluble phenolic acid levels in peel to determine associations with disease levels.

MATERIALS AND METHODS

'Hass' avocado fruit was harvested from trees in commercial orchards in northern NSW determined to have 'high' or 'low' crop loads, in 2007 and 2008. Peel samples were collected from sub-samples for cation analyses, and at harvest, 'sprung' (when fruit first start to soften) and 'eating ripe' for total soluble phenolic acid contents. Other fruit samples were maintained in a controlled environment room (22–23°C, 65% RH), and assessed at eating ripe stage for anthracnose and stem end rot diseases. Dried peel samples were finely ground and analysed for major cations by SGS Agritech. Phenolic acid contents were determined by using the Folin-Ciocalteu reagent on samples extracted with 50% v/v methanol, and compared against a gallic acid standard curve.

RESULTS AND DISCUSSION

In both years, the incidence of fruit with anthracnose disease was significantly less when harvested from trees with high crop loads (Table 1). Severity of disease was also less, but not significantly. Conversely, stem end rot, caused primarily by *Botryosphaeria* spp., was more severe in fruit from high crop bearing trees (significant in 2008, Table 2). The trees were drought stressed, which is thought to exacerbate stem end rot diseases in mango and avocado, and the greater crop load most likely added to this stress. There was a higher percentage of marketable fruit from high crop load trees (data not shown).

Table 1. Severity and incidence of anthracnose in "Hass" fruit from high and low crop bearing trees in 2007 and 2008

Crop load	% severity anthracnose		% incidence anthracnose	
	2007	2008	2007	2008
High	11.1	34.2	27.1 b	78.8 b
Low	19.5	53.6	50.3 a	90.0 a

Table 2. Severity and incidence of stem end rot in "Hass" fruit from high and low crop bearing trees in 2007 and 2008

Crop load	% severity stem end rot		% incidence stem end rot	
	2007	2008	2007	2008
High	10.5	3.83 a	33.5	16.5
Low	4.8	1.42 b	27.7	8.8

Cation analyses show that peel from fruit harvested in 2008 from high crop load trees had significantly higher calcium, lower N:Ca ratio, and higher Ca+Mg:K ratio than from fruit from low crop load trees. This is consistent with what was previously known, ie. that high Ca and low N is associated with better quality fruit (2).

Table 3. Effect of crop load on major cations and their ratios in 'Hass' avocado peel at harvest, 2008

Crop load	Content (% dry wt of fruit peel)				N:Ca ratio	Ca+Mg:K ratio
	N	Ca	Mg	K		
High	0.792	0.036 a	0.082	1.078	22.3 b	0.110 a
Low	0.868	0.028 b	0.078	1.232	31.8 a	0.087 b

The results for soluble phenolics in these trials did not indicate that they were influenced by crop load, and no clear associations can be made between severity and incidence of postharvest disease and total soluble phenolic acid content. There was, however, a clear association between phenolics and disease reaction and rootstock type in another study (unpublished).

Fruit quality can thus be improved by optimising tree yield and nutrient concentrations, and reducing drought stress.

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64 Hosts of citrus scab, brown spot and black spot in coastal NSW

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INTRODUCTION

Characterisation of endemic pathogens, including knowledge of host range and pathotyping, is important for disease control programs and trade negotiations.

Citrus scab is a serious leaf and fruit disease of lemons in coastal areas of NSW and Qld. Previous studies have reported six *Elsinoë fawcettii* pathotypes on citrus worldwide (1, 2). In Australia, the Tryon's and "Lemon" pathotypes have been described (1) but the pathotype range may not have been well-represented as all of the isolates studied were from one lemon producing area of NSW.

Citrus brown spot (*Alternaria alternata*) affects fruit and foliage of mandarins, tangelos and tangors in the humid coastal regions of Australia and is rarely found on grapefruit. In Florida, isolates sampled from grapefruit and the hybrid cv. Nova were genetically distinct from isolates sampled from other hybrid cultivars including Minneola tangelo and Murcott tangor (3).

Citrus black spot (*Guignardia citricarpa*) is a serious disease of Valencia and navel oranges in coastal areas of eastern Australia. A non-pathogenic species *G. mangiferae* has a wider host range which includes citrus.

The aim of this study was to observe the incidence of scab, brown and black spots in an old citrus germplasm collection containing some rare species. The trees had not been sprayed for several years. By expanding the host varieties observed additional pathotypes may be found.

MATERIALS AND METHODS

Surveys were conducted in 1999 and 2009 in a citrus arboretum at NSW DPI's Gosford Horticultural Institute. Symptoms on fruit and foliage were recorded for scab, black spot and brown spot. Further work will be conducted to characterise and identify pathotypes.

RESULTS AND DISCUSSION

Survey findings are presented in Table 1. Citrus scab was not observed on Satsuma mandarin, in contrast to overseas studies (2). Wotton rough lemon was the only rough lemon clone not to show symptoms of scab. Leaves of this clone are typical of rough lemon, but the fruits are acharacteristic, suggesting that it is a hybrid.

The surveys found no evidence of brown spot affecting grapefruit, even though symptoms have been seen on a red grapefruit tree in Qld adjacent to badly affected Minneola tangelo trees.

Sour orange is reportedly not susceptible to black spot, but one clone of smooth seville (a sour orange hybrid) in the arboretum showed symptoms. Lemons are often a preferred host of *G. citricarpa* with infections in new regions often occurring first on lemons. Infection was severe on the foliage of a number of lemon varieties and hybrids and on the rootstocks Troyer citrange and Swingle citrumelo, but not on *C. trifoliata*. All species/clones except *C. trifoliata* showed melanose (*Diaporthe citri*) symptoms to varying degrees including the native finger lime *C. australasica*.

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Table 1. Survey findings for scab and other fungal pathogens in the citrus arboretum, Narara NSW

Citrus species ¹	Common name	Varieties with scab symptoms observed	Varieties with brown spot symptoms	Varieties with black spot symptoms
<i>C. reticulata</i> Blanco	mandarin	tangerine ex China	Shekwasha, Szinkom, Ladu, Cleopatra, Batanges, Emperor, Sunki, Satsuma	Cleopatra, Batanges
<i>C. x microcarpa</i> Bunge	calamondin		calamondin	
<i>C. x insitorum</i> Mabb	citrange			Troyer citrange, Swingle citrumelo
<i>C. x aurantium</i> L. (= <i>C. aurantium</i> L. and <i>C. sinensis</i> (L.) Osbeck)	sour, sweet, Valencia and navel oranges, grapefruit & King orange	Taiwanica	tangelo (Sampson, Minneola, Orlando, Yalaha, San Jacinto, Wekiwa, Seminole, Thornton, Sexton)	smooth Seville (Waddell), San Jacinto tangelo, Shunkokan
<i>C. x limon</i> (L.) Osbeck	lemon	lemon (Volkamer, Lisbon, Eureka, Lemonade, Yen Ben, Villafranca, Thornless, Meyer, Assam), Rangpur lime		lemon (Volkamer, Lisbon, Eureka, Lemonade, Yen Ben, Villafranca, Thornless, Meyer), Rangpur lime
<i>C. x taitensis</i> Risso (= <i>x jambhiri</i> Lush.)	rough lemon	rough lemon (Settree, Wilson, Narara)	rough lemon (Settree, Wilson, Narara, Wotton)	rough lemon (Settree, Wilson, Wotton)
<i>C. x aurantiifolia</i> (Christm.) Swingle	lime	lime (West Indian, Kusiae, acid, accession 3233)	Kusae lime	
<i>C. x indica</i> Tanaka	Indian wild orange	Indian wild orange		

1. Classification is according to Mabberley DJ (1997) A classification for edible citrus. *Telopea* **7**, 167-720

65 Nitrogen form affects *Spongospora subterranea* infection of potato roots

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INTRODUCTION

Powdery scab of potato tubers (*Solanum tuberosum*) is caused by the plasmodiophorid pathogen *Spongospora subterranea* f. sp. *subterranea*. The disease is important where potatoes are grown under intensive management, as it causes severe reductions in quality of seed and ware potatoes from affected crops (1). The pathogen can also infect potato roots, causing root galls and reducing plant growth (1). Manipulation of soil nutrients could be part of integrated powdery scab management. Nitrogen (N)-containing amendments have been shown to reduce (2) and increase (3) powdery scab in field-grown potatoes.

We present results from an experiment that aimed to determine effects of different rates and types (nitrate or ammonium) of N compounds on infection of potato plant roots by *S. subterranea*.

MATERIALS AND METHODS

The experiment was carried out in a glasshouse compartment (17°C ± 2°C; 16 h light, 8 h dark). Tissue-cultured potato plantlets (cv. Iwa; very susceptible to powdery scab) were planted into a 50:50 w:w mix of field soil and coarse sand (>1 mm) in plastic pots (11 cm diam., 680 ml capacity). The soil in each pot was irrigated with deionised water (by weight) to 90% water holding capacity three times each week for 8 weeks.

Two weeks after planting, treatments of three N compounds (ammonium nitrate (NH₄NO₃), ammonium sulphate ((NH₄)₂SO₄), calcium nitrate (Ca(NO₃)₂·4H₂O)) were each applied to separate pots as solutions at five different rates, calculated to apply equivalent amounts of N. The rates were 0.05, 0.10, 0.20, 0.40, or 0.60 g N pot⁻¹, equivalent to 62.5, 125, 250, 500 and 750 kg N ha⁻¹. At the same time the pots were each inoculated with suspensions of *S. subterranea* sporosori (30,000 pot⁻¹). Two control treatments of no added N with or without inoculum were also applied. The experiment included 17 treatments (three N compounds, five rates of each, plus two controls), and was of randomised complete block design with seven replicates.

The plants were harvested 8 weeks after planting. Each plant was carefully washed free of soil, the number of *S. subterranea* root galls was counted and root dry weight (10 h at 70°C) determined. Data were transformed (square root) to stabilise variances and analysed with ANOVA.

RESULTS AND DISCUSSION

Fig. 1 summarises data of severity of *S. subterranea* galling on the roots of harvested plants. No galls were observed on uninoculated plants, while plants from the nil N inoculated treatment had a mean of 80.5 galls g⁻¹ root. All of the N treatments reduced root galling. Increasing rates of N for both ammonium sulphate and ammonium nitrate gave decreasing numbers of root galls. For calcium nitrate, however, increasing rate had little effect on root galling. At the three lowest rates in N, of the three N-containing compounds, ammonium sulphate gave the greatest reduction in root galling.

These results indicate that ammonium-N is more inhibitory to *S. subterranea* infection of potato roots than nitrate-N. They also

suggest that increased powdery scab in the field after addition of high rates of N fertiliser (3) were unlikely to be due to direct effects of N on the pathogen, but may have been caused by indirect host growth effects (e.g. increased root mass resulting in increased amounts of zoospore inoculum).

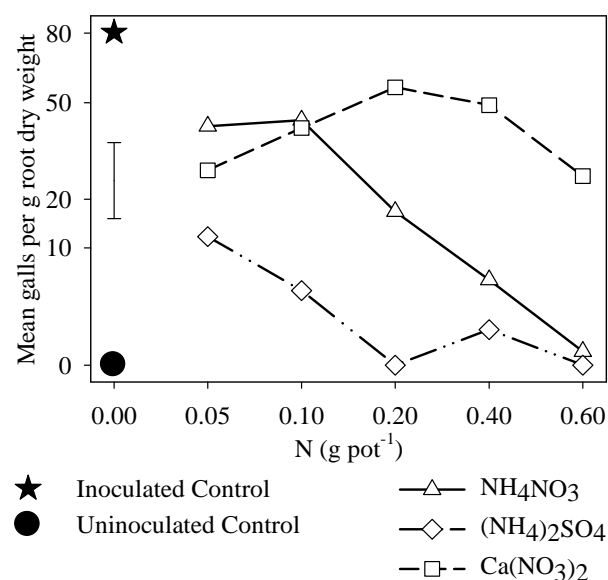


Figure 1. Mean numbers of *Spongospora subterranea* root galls on potato plants grown in pots treated with different amounts of N-containing compounds. Bar = LSD (P=0.05) for visual comparison of the means (square root transformed scale).

Ammonium-N is usually converted to nitrate by soil micro-organisms soon after application (4). It is likely, therefore, that the inhibitory effect of ammonium on *S. subterranea* occurred during early host infection stages, possibly affecting zoospore release from sporosori and/or infection of host roots.

These results suggest that ammonium-N may usefully reduce *S. subterranea* infection of potato. This should be confirmed in field evaluations in crops of potatoes grown in soil naturally infested with the pathogen.

ACKNOWLEDGEMENTS

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

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66 Relationships between *Spongospora subterranea* DNA in field soil and powdery scab in harvested potatoes

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INTRODUCTION

Powdery scab (caused by *Spongospora subterranea* f. sp. *subterranea*) is an important disease of potato (*Solanum tuberosum*). This disease is difficult to control, partly because *S. subterranea* can survive in soil for many years (1). Molecular detection and quantification of the pathogen in soil are possible components of disease management, to indicate pre-planting *S. subterranea* inoculum levels and powdery scab risk (1).

We measured *S. subterranea* DNA levels in soil from a naturally infested field at planting and powdery scab in subsequently harvested tubers, over two growing seasons. Relationships between pre-planting soil DNA and disease on harvested tubers were examined.

MATERIALS AND METHODS

The field area (0.36 ha) for this study had been previously used as a trial during the 2006/07 growing season. Twelve treatments (two cropping histories, three nitrogen fertiliser application rates, two irrigation regimes) were applied to potatoes grown in 96 plots, each 5 × 5 m. The trial was of split split plot design with eight replicates (2). The treatments resulted in different levels of powdery scab in each plot (April 2007). During two subsequent growing seasons (2007/08 and 2008/09), the same plots were planted with cv. Agria (very susceptible to powdery scab) in October, in rows (2 m long; eight tubers/row) centrally in the 5 × 5 m plots. Soil samples were taken from each row at planting and analysed for *S. subterranea* DNA, using quantitative PCR techniques (3). Resulting tubers were harvested in April, washed free of soil and individually assessed for powdery scab severity (0 = no disease, 1 = 5% tuber surface affected, 2 = 20%, 3 = 46%, 4 = 60%). Relationships between the *S. subterranea* DNA in soil and powdery scab incidence and severity were explored graphically and with linear correlations (Pearson's *r*).

RESULTS AND DISCUSSION

2007/08 growing season. Powdery scab incidence in the plots varied from 30 to 100%, and mean severity score varied from 0.3 to 2.7 (equivalent to 2 to 40% of tuber surface area affected). The relationships between *S. subterranea* DNA quantities in soil and powdery scab incidence ($r = 0.53$) and severity ($r = 0.63$) are illustrated in Figure 1.

2008/09 growing season. Powdery scab incidence in the plots varied from 4 to 83%, mean severity score varied from 0.04 to 1.6 (equivalent to 0.2 to 16% of tuber surface area affected) and amount of *S. subterranea* DNA varied from 58 to 1997 pg g⁻¹ soil. The relationships between amount of DNA in soil and powdery scab incidence and severity were very poor ($r = 0.02$ and 0.07 respectively).

This study has shown moderate to poor correlations between *S. subterranea* DNA in soil sampled at the time of sowing and powdery scab in harvested tubers. These results were from a field where soil DNA quantities and powdery scab were assessed from 96 evenly spaced positions in a 0.36 ha area (≈ 270 samples

per ha.). In this study, where large pre-planting quantities of *S. subterranea* DNA occurred in soil, DNA quantification did not accurately predict incidence or severity of powdery scab in harvested tubers.

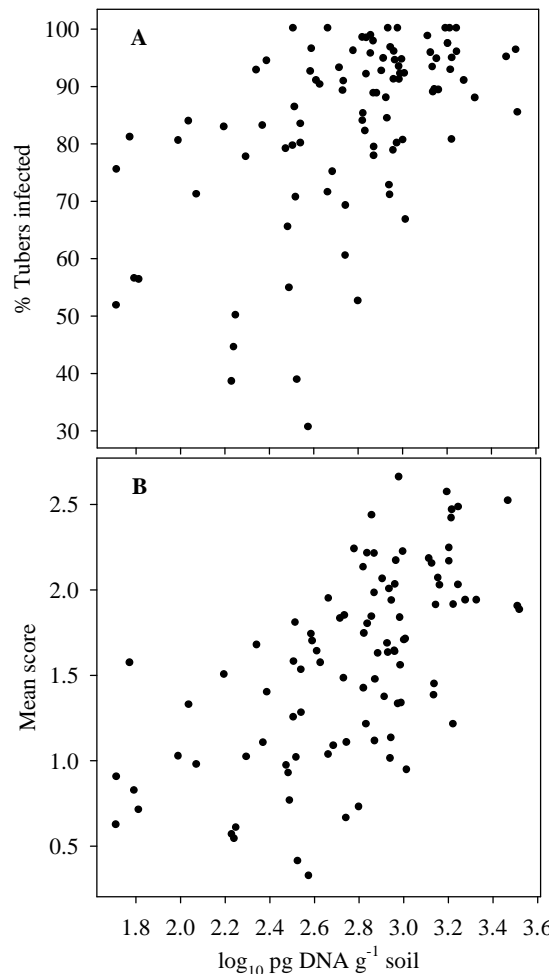


Figure 1. Relationships between *S. subterranea* DNA in soil at planting and powdery scab incidence (A) and mean severity score (B) for potatoes grown in field plots.

ACKNOWLEDGEMENTS

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

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6 Bacterial canker of tomato: Australian diversity of *Clavibacter michiganensis* subsp. *michiganensis*

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INTRODUCTION

Bacterial canker of tomato is an important disease in Australian tomato production, especially amongst the greenhouse industry. The disease is caused by systemic vascular infection of the bacteria *Clavibacter michiganensis* subsp. *michiganensis* (Cmm). Canker infection can result in yield reductions of up to 100%.

Currently there are no effective chemical or biological control for canker, the only effective methods are the quarantine and eradication of infected material. The external symptoms of bacterial canker have altered over the past decades. Whether this is due to the use of newer tomato cultivars which react differently to infection or due to the introduction of new genetic strains of the bacteria is not known.

It is possible that there are new strains present in Australia since Cmm can be seed borne and imported seed is generally untreated since the relaxation of Australian quarantine requirements in the early 1990s.

MATERIALS AND METHODS

Isolate collection. Isolates were collected from tomato growing areas around Australia with a particular focus on greenhouse tomatoes. International isolates have been sourced from the Belgium culture collection (BCCM) and from America.

Genetic diversity. DNA was extracted from the isolates using the Qiagen DNeasy kit, before quantification and dilution. DNA fingerprinting was undertaken using the ERIC, BOX and REP PCR (1). Further analysis of the genomic internal transcribed region (ITS) was undertaken on all isolates using a combination of sequencing and PCR-RFLPs (2).

Pathogenic diversity. A range of tomato cultivars were used to compare pathogenicity of Cmm isolates selected based upon the genetic diversity results. Isolates were also screened against other solanaceous crop plants commonly grown including eggplant and capsicum.

RESULTS

Genetic diversity. Preliminary screening results using BOX, ERIC and REP primers have revealed differences amongst Australian Cmm isolates. Sequencing analyses of the ITS region of four selected isolates has shown some base changes allowing the development of PCR-RFLP.

Pathogenic diversity. All tomato cultivars examined showed high levels of susceptibility to Cmm, though symptom expression appears to be cultivar dependent. Of the isolates examined the majority were pathogenic, though there was at least one isolate which appears to be avirulent.

Experiments examining a wider host range of Cmm revealed that pathogenic isolates were able to infect the two capsicum cultivars examined resulting in small localised lesions on the leaf lamina where the inoculum was initially applied. No systemic infection was observed within the capsicum plants. No symptoms were observed on the eggplant cultivar used.

DISCUSSION

Cmm diversity. Early results from the DNA fingerprinting of the Australian isolates of Cmm has revealed genetic diversity. Further comparisons with international isolates and isolates from field-grown tomatoes will help understanding of whether this diversity is reflected in the international diversity or localised population drift potentially due to the high selection pressure within the greenhouse environments.

Preliminary analyses of the genetic diversity of the avirulent isolate has not revealed any distinct differences. The avirulent isolate is being further tested using pulse field gel electrophoresis to examine the presence of pathogenicity conferring plasmids and virulence genes previously described (3).

Only limited pathogenic diversity has been observed amongst isolates of Cmm. Although there was some variation between tomato cultivars in symptom expression, all tomato cultivars assessed showed high levels of susceptibility to the majority of isolates and eventually died due to application of Cmm. The localised lesions which developed on the capsicum leaves did not spread systemically in these experiments, implying that in a controlled environment only direct contact with Cmm on the leaves will lead to disease. Further testing using Cmm isolates isolated from capsicum plants will be undertaken to determine whether more severe disease could develop.

ACKNOWLEDGEMENTS

This work was funded by ACIAR, NSW DPI, Horticulture Australia Ltd, AusVeg and the tomato consortium. Acknowledgement for technical assistance from J. Collins, K. Turton, and S. Austin.

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67 Detection of *Mycosphaerella fijiensis* in the skin of 'Cavendish' banana

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INTRODUCTION

Mycosphaerella fijiensis, cause of black leaf streak, has a very slow incubation period compared with many other pathogens. The fungus infects leaves as they emerge from the plant. The first symptoms (rusty streaks) appear in the highly susceptible 'Cavendish' types in 2–3 weeks, extending to over 35 days for the more resistant 'Ducasse', 'Pahang' and 'Pisang Mas'. In some genotypes symptoms may not be expressed until the leaf begins to senesce (1). These patterns of host response show that the pathogen has the ability to survive without symptoms in the leaf for extended periods. There are no records of infection of the fruit of dessert bananas. A record of infection in plantain (2) shows that the fungus has the capacity to invade the skin of fruit. This study aimed to investigate whether the fungus can be present without symptoms in the skin of 'Cavendish' bananas.

MATERIALS AND METHODS

Source of fruit. Green, fully developed fruit were obtained from diseased 'Cavendish' plants in the field in Samoa. Additionally, 'green-mature' and ripe fruit were obtained from the local market.

Isolation protocol. Pieces of skin tissue approximately 5 mm square and 0.5–1.0 mm thick were excised aseptically and plated onto Potato Dextrose Agar (PDA) or V8 agar, both modified with streptomycin and penicillin (100 µg/ml of each). Plates were incubated under continuous white/near-UV light and were examined microscopically after five days

Overall, a total of 1040 skin pieces were taken from 60 fruit from 13 different sources. Five pure cultures of putative *M. fijiensis* were returned to New Zealand under a Biosecurity New Zealand permit to confirm their identities.

Identification of isolates. The identities of the cultures returned to New Zealand were confirmed by spore morphology, polymerase chain reaction (PCR) using species-specific primers provided on a confidential basis by the Cooperative Research Centre (CRC) for Tropical Plant Protection, and by the sequencing of the internal transcribe spacer region of rDNA.

RESULTS AND DISCUSSION

Of the five fruit isolates returned to New Zealand, two were positively identified as *M. fijiensis*, with concurring results from spore morphology, PCR, and sequencing (100% homology to sequences of *M. fijiensis* in GenBank). PCR results are shown in Figure 1. Both isolates were obtained from different skin samples from the same fruit. Microscopical examination (in Samoa) of a cluster of hyphae on skin of a different fruit revealed dark hyphae strongly resembling the early stages of a stroma of *M. fijiensis*. The structure was insufficiently developed to obtain a positive identification based on morphology, and overgrowth by other fungi prevented its isolation into pure culture. In all three cases, (two confirmed *M. fijiensis*, one suspected), the fungus was associated with minute (~1 mm diameter) red, necrotic flecks on the surface of the skin. Red flecks were relatively common on many of the test fruit. Most did not yield any fungi and others were overgrown by various fungal species before the slow growing *M. fijiensis* would have had time to emerge. It cannot be determined from this study whether there

is a constant association between the red fleck symptom and *M. fijiensis*.

In many cases the presence of *M. fijiensis* may have remained undetected because of overgrowth of the skin pieces by other faster growing fungi such as *Colletotrichum* spp., *Cordana musae*, *Penicillium* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Rhizopus* sp., *Trichoderma* sp., and *Pestalotiopsis* sp.

This study has shown that *M. fijiensis* can infect and survive without symptoms in the skin of 'Cavendish' banana. While only a very low recovery rate was achieved in this study, the incidence in nature may be much higher.

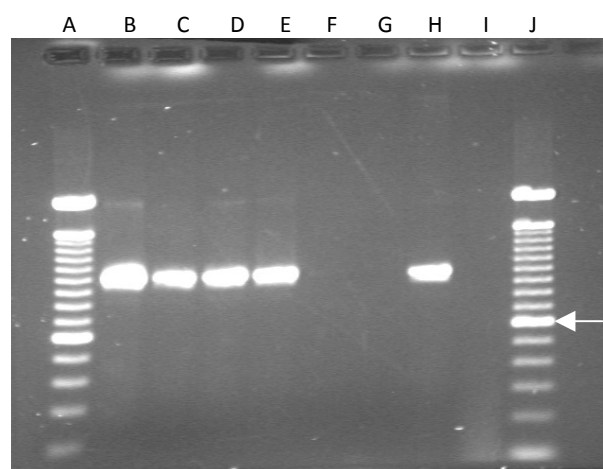


Figure 1. Amplified *Mycosphaerella fijiensis* products of approximately 1050 bp. Lane A: 100 bp ladder B: 88a; C: 88b; D: Mfb; E: Mfc; F: Myc#1; G: 589 yellow Sigatoka; H: 748 *M. fijiensis* (positive control); I: blank (negative control); J: 100 bp ladder. Arrow indicates 600 bp on 100 bp ladder.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Samoa Ministry of Agriculture, Fisheries, Forests and Meteorology for access to the laboratories of the Nuu Crop Research Centre, and assistance in this study. We thank Dr Juliane Henderson of CRC for Tropical Plant Protection for supplying primer sequences.

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46 Two new books: *Diseases of Fruit Crops in Australia* and *Diseases of Vegetable Crops in Australia*

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Accurate information on disease diagnosis and management is essential for sustainable crop production. Two new books, *Diseases of Fruit Crops in Australia* and *Diseases of Vegetable Crops in Australia*, provide comprehensive coverage of important diseases affecting the broad range of fruit and vegetables grown throughout Australia. Written in a practical, straight forward style, the text explains how to identify and manage each disease, describing the symptoms of the disease, its importance, the means of infection and spread, and disease management.

Based on the highly regarded early 1990 editions of *Diseases of Fruit Crops* and *Diseases of Vegetable Crops* published by the Department of Primary Industries and Fisheries, Queensland, these new books have been extensively revised and expanded. Emphasis is placed on integrated disease management and diseases that are biosecurity threats to Australian fruit and vegetable production.

The text is supported by quality colour images. The books will become new standard references in applied plant pathology in Australia for fruit and vegetable crops.

FEATURES

- Chapters are authored by experienced plant pathologists from throughout Australia.
- Written in a straightforward style with a minimum of scientific terms.
- Provides accurate information about significant diseases affecting major and specialty fruit crops and vegetable crops in Australian tropical and temperate regions.
- Each disease is extensively illustrated with high quality colour photographs.
- Contains a comprehensive glossary and provides up-to-date sources of further information.
- Describes key exotic diseases that are biosecurity risks to Australian fruit and vegetable growers.

Both books are being published by CSIRO Publishing (Landlinks Press) and are due for release in October 2009

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71 Infection and host responses in interactions between melon and *Colletotrichum lagenarium*

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INTRODUCTION

Melon is an economically important horticultural crop that is susceptible to anthracnose caused by *Colletotrichum lagenarium*. Two types of infections are caused by *Colletotrichum* species: intracellular hemibiotrophic invasion or subcuticular intramural necrotrophic invasion (1). However, the infection process of melon anthracnose caused by *C. lagenarium* remains unknown. This study of the compatible interaction between *C. lagenarium* and melon leaves investigated the infection process and monitored defence responses of the melon plant.

MATERIALS AND METHODS

Preparation of infected tissues. Seeds of rockmelon cv. Galaxy and Ultra sweet Miami (Terranova seeds Pty Limited, NSW, Australia) were grown in 10 cm plastic pots filled with UC potting mix in the glasshouse at 24°C, with illumination for 16 h. Plants were watered daily and fertilised with Aquasol® weekly. Three week old seedlings were inoculated on the abaxial surface of the first leaf with a suspension of 10^6 conidia mL⁻¹ of *C. lagenarium*. Inoculated plants were maintained at 25°C, 100% relative humidity for 24 h, then returned to the glasshouse.

Light microscopy. Leaf samples were collected at 6, 12, 24, 48, 72, 96 hrs after inoculation. Decolourised sections were immersed in lactophenol for 1min and then stained with 0.025% aniline blue for 30 min. After staining, the tissues were rinsed (2|2min) in lactophenol and mounted in fresh lactophenol on glass slides for microscopy(2). Callose was visualized under UV after staining with aniline blue (3).

RESULTS AND DISCUSSION

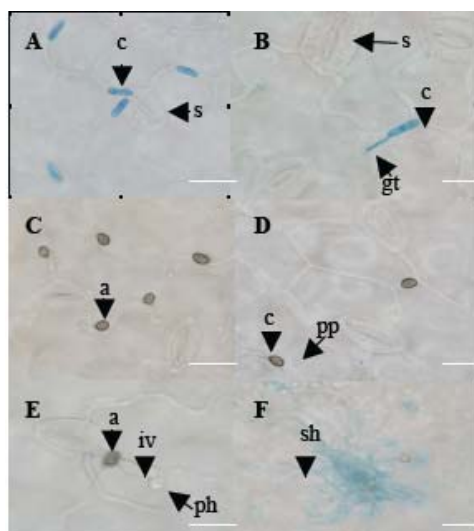


Figure 1. Infection structures of *C. lagenarium* in the first leaves of rockmelon. (A) Ungerminated conidia on the leaf surface with stomata 6 hai. (B) Conidia with germ tubes 12 hai. (C) Melanised appressoria 24 hai. (D) Appressorium with penetration peg 48 hai. (E) Formation of infection vesicle and primary hyphae 72 hai. (F) Formation of secondary hyphae 96 hai. c=conidia;s=stomata; gt=germ tube; a=appressorium; pp=penetration peg; ph=primary hyphae;sh=secondary hyphae; iv=infection vesicle;hai=hour after inoculation.Bars=20µm.

Conidia attached and germinated on the leaf surface 6 hai (Fig. 1A), and differentiated a germ tube at one tip 12 hai (Fig. 1B). Melanised appressoria were first observed 24 hai, sometimes formed directly from one tip of the conidium (Fig. 1C), or from the tip of the germ tube. Penetration pegs were observed 48 hai (Fig. 1D). By 72hai, epidermal cells of melon leaves had been penetrated and contained intracellular fungal structures comprising swollen, saccate infection vesicles with elongated neck regions (Fig. 1E). Infection vesicles enlarged and formed primary hyphae (Fig. 1E), and at this stage of host-pathogen interaction, infected melon leaves were symptomless. Beyond 72 hai, secondary hyphae developed from the primary hyphae and invaded surrounding tissues (Fig. 2F), and the infected melon leaves developed visible anthracnose symptoms. The results also indicated that the resistant and susceptible cultivars use the same infection process.

Callose deposition around the infection sites was noted 48 hai in susceptible and resistant cultivars (Fig. 2, Fig. 3). Callose deposition was brighter and more intense in the resistant cultivar.

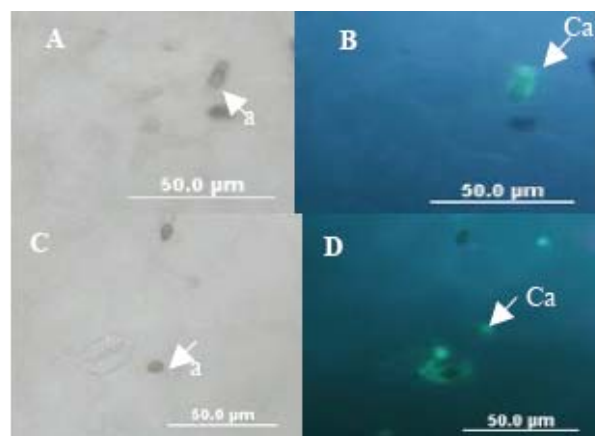


Figure 2. (A) Light and (B) UV micrographs showing the accumulation of callose 48 hai of the first leaves of susceptible melon with *C. lagenarium*. (C) Light and (D) UV micrographs showing the accumulation of callose 48 hai of resistant melon.

a=appressorium; Ca=callose

These results indicate that the infection process of *C. lagenarium* was intracellular hemibiotrophic invasion.

ACKNOWLEDGEMENTS

This research was supported by the Australian Centre for International Agricultural Research (ACIAR). We thank Suneetha Medis for technical assistance.

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70 Disease-management strategies for the rural sector that help deliver sustainable wood production from exotic plantations

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INTRODUCTION

A training workshop and post-workshop field trip was held in May 2009 in Indonesia to advance knowledge and understanding in disease management. It was opened by the Minister for Forestry and attended by a wide range of participants from the forest, oil palm and rubber industries, universities, and research and government agencies. The workshop was supported by international experts from South Africa, the United Kingdom and Australia.

Case studies were used at the workshop to provide training and exposure for participants in concepts of forest pathology, biosecurity and forest health surveillance and their application towards developing strategies for disease management. These case studies focused on disease issues and threats of immediate relevance to tree crops in Indonesia, for example, fungal rot in hardwood plantations, rubber and oil palm, rust galling in *Paraserianthes falcata*, and the significance of a guava rust incursion. An exercise was carried out in the field to train in the basic concepts of ground based forest health surveillance. The field trip in Sumatra examined demonstration sites and experiments in acacia and eucalypt areas most severely affected by root rot, and included hands-on experience in disease assessment and novel ways to examine disease risk.

OUTPUTS OF TRAINING EXERCISE

Position paper. The position paper focuses on using all available information about root and basal stem rot in plantation-based industries to assist in capturing both the current status of forest disease management capacity in Indonesia and what type of capacity will be required to combat some very serious diseases of plantation crops. It first considers the background that has led to root rot and basal stem rot becoming diseases that have a significant economic effect on plantation-based industries. A summary of the current size of the plantation estates in the oil palm, pulpwood and rubber industries is then provided. These sections give us an idea of the possible returns from investing in building capacity.

The paper then collates information that has been collected from professional staff working in both the private and public sector in roles that are connected to disease management for oil palm, forestry (primarily pulpwood) species and rubber, and supports this information with that from published literature. A separate section considers the concept of ecosystem management—this research focus lies primarily in the public sector. Next there is a dissertation on biological control that examines the potential characteristics and development of control agents and the challenges that must be overcome to make them work. These three sections assist in highlighting the types of research and operational disease management capacity required.

The current capacity to deliver professional services in disease management is then examined. The paper concludes with a consideration of the part of Indonesia's higher education system that delivers training in Plant Protection and Plant Pathology and

policy directions that are relevant to both this education and the application of disease management.

Proceedings and DVD *Disease Management Strategies in Plantations*. The Proceedings will summarise the information from the workshop from the various sessions (Introduction to Disease Management; Morphological and Molecular Identification Tools; Forest Health Surveillance; Biosecurity; Chemical, Genetic and Biological Control; Silvicultural and Risk Management; Integrated and Ecosystem Management; Policy Development).

The DVD which contains all the talks from the Workshop is available on request and the Proceedings will be available in September.

Field guide. A field guide to the identification of basidiomycete root rot diseases in tree crops will be published at the end of 2009. This will include crown and root symptoms associated with the various stages of root rot disease and a description of the sporocarps associated with the various fungal pathogens capable of causing root rot disease.

SUMMARY

As in Australia there is little specific University training in forest pathology or disease management. Plant pathology education is comparatively well resourced in Indonesia, especially in Bogor, and the industries can draw from this pool of graduates. Barriers to building expertise in forest disease management lie in the fact that young people do not wish to live in remoter regions and there are often organisational barriers to the sharing of expertise and a collaborative approach to solving problems, even within the same industry.

The Government of Indonesia has no formal approach to disease management in its forest policy. However the Minister has acknowledged the problem of disease, especially root-rot disease (which is probably the most serious pest problem faced by the hardwood plantation industry) and is actively encouraging a cooperative approach to disease management. Substantial funding is potentially available to promote this collaboration and this supports the case for more open communication as was achieved by the workshop and field trip. Root rot disease has been a surprising catalyst for opening pathways of communication.

ACKNOWLEDGEMENTS

We thank the AUSAID Public Sector Linkage Programme for funding this activity. We also thank the many numerous Indonesian colleagues who participated in this activity.

68 Rapid and robust identification of fungi associated with *Acacia mangium* root disease using DNA analyses

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INTRODUCTION

Indonesia, like many developing and developed countries, lacks people with experience in identifying root rot pathogens, both as sporocarps but more particularly, in culture. This increases the difficulty of managing *Acacia mangium* plantations which suffer severe economic losses due to root rot. This type of disease is caused by a variety of primary pathogens in Indonesian plantations. Existing forestry research laboratory facilities already use DNA techniques in plant genetics research and this capability was adapted to identification of fungi isolated from diseased roots of *Acacia mangium*.

Several species of *Ganoderma* are associated with root rot in *Acacia mangium* (1). As part of ACIAR project FST/2003/048 a large number of fungal cultures were isolated from the roots of *A. mangium* and sporocarps collected from *A. mangium* plantations, with a view to determining the most prevalent and damaging root pathogens, elucidating their mode of dispersal and developing strategies for their management. Accurate isolate identification is a prerequisite for success in these aims.

MATERIALS AND METHODS

DNA was extracted and the rDNA ITS was amplified and sequenced (1). Isolates were grouped into Operational Taxonomic Units (OTUs) based on DNA sequence similarity. The OTU was identified by DNA sequence identity with herbarium collections where possible.

Development of species-specific primers targeting the rDNA ITS allowed faster and cheaper identification of two of the most prevalent species associated with root rot in *Acacia mangium*, *Ganoderma philippii* and *G. mastoporum*. Subsequent to the development of these specific primers all isolates isolated from roots or sporocarps were screened with these primers. This allowed faster identification and reduced the number of isolates sent for sequencing.

RESULTS AND DISCUSSION

Over 200 root rot isolates were confirmed as *G. philippii* or *G. mastoporum* either by DNA sequencing or species-specific PCR (Figure 1). Another 120 cultures were grouped into 43 operational taxonomic units (OTUs) by DNA sequence similarity.

Eighteen OTUs were linked by DNA sequences to sporocarp collections, facilitating the morphological verification of culture identification.

Identification of the remaining OTUs is based on DNA sequence similarity to sequences from public DNA databases. Only one *Ganoderma* isolate has not been linked to a sporocarp collection. 33 OTUs are linked to species/genus information in public DNA databases, providing an indication, at various taxonomic levels of species affinities.

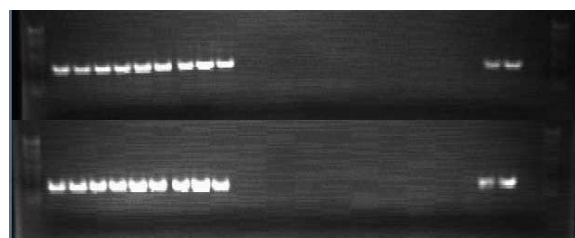


Figure 1. Species-specific PCR for identification of *Ganoderma philippii*. Upper panel, PCR with primers Gphil2f/Gphil6r; lower panel, PCR with primers Gphil3f/Gphil4r. Lanes contain: 1, DNA size marker; 2–10, *G. philippii* isolates; 11–22, other *Ganoderma* spp.; 23–24, positive controls (*G. philippii*); 25, negative control (no DNA).

G. philippii and *G. mastoporum*, *G. aff. australe*, *G. aff. steyaertanum*, *G. subresinosum*, *G. aff. subresinosum*, *G. colossium*, *G. weberianum*, *Amauroderma rugosum* and *Phellinus noxius* were isolated from *A. mangium* plantations. Isolates from diseased roots are predominantly *G. philippii*, with a low incidence of *G. mastoporum* and *Phellinus noxius*.

Sporocarps of *Fomes*, *Irpex*, *Phlebia*, *Trametes* spp. have been collected and formally identified by DNA analysis. This study also discovered another fungal species that warrants investigation as a potential root-rot biocontrol. Some cultures from roots were identified as belonging to the genus *Phlebiopsis*. *Phlebiopsis gigantea* has been demonstrated to be an effective prophylactic biocontrol for root rot caused by *Heterobasidion annosum*.

Maintaining a large number of fungal isolates in tropical regions poses a challenge. Confident and rapid identification of fungal isolates has reduced the work required to maintain fungal isolates by allowing non-target fungi to be discarded. This also reduces the risk of culture contamination by 'weedy' species.

ACKNOWLEDGEMENTS

This work was funded by ACIAR project FST/2003/048.

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69 Survey of the needle fungi associated with Spring Needle Cast in *Pinus radiata*

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INTRODUCTION

Spring needle cast (SNC) is currently classified as a serious disease of *Pinus radiata* growing in closed-canopy stands on high altitude, wet sites in Tasmania (1). SNC affects about 30% of the *Pinus radiata* estate in Tasmania and causes the premature casting of needles at the end of their first year. This leads to growth reductions in direct proportion to the amount of defoliation. Stands with moderate or severe SNC can be expected to suffer potential losses in clearfall volume of 30–50% (1). Unlike other serious needle cast diseases elsewhere in Australia and New Zealand such as *Dothistroma septospora* or *Cyclaneusma*, SNC in Tasmania is not considered to be a classical needle blight disease caused by a primary fungal pathogen. It is thought to be caused by a suite of endophytic fungi that are triggered into secondary pathogenic activity by an environmental stress. Three fungal species are considered to play a role in Spring Needle Cast in Tasmania: *Cyclaneusma minus*, *Lophodermium pinastri* and *Strasseria geniculata* (2).

The *Pinus radiata* Spring Needle Cast Marker Aided Selection (MAS) trial was planted in June 1999 by Forestry Tasmania in Oonah, North West Tasmania (annual rainfall: 1655mm/yr; mean daily temperature: 9.9 °C; altitude 450 metres). There are three full sib families with known breeding values for SNC (3).

The objective of this study was to characterise the fungal communities associated with needles on trees scored for SNC damage in the MAS trial.

MATERIAL AND METHODS

The needle samples were collected in spring 2007 from the Oonah SNC MAS trial. The trees in this trial were scored for SNC severity immediately before sample collection. Trees were given a score ranging from 1 (no disease) to 4 (severe disease). For each disease score within each family, 3 trees were sampled for needles. Three different ages of needle were collected from a tree.

DNA was extracted from needles and fungal DNA was amplified by PCR (4). PCR products from the same aged needles of trees in the same disease class and family (i.e. 3 trees) were pooled then cloned using a commercial kit. Thirty-two colonies from each cloning reaction were randomly selected and screened using PCR-RFLP to reduce the number of samples for sequencing. Approximately 12–16 clones from each set were sequenced. Sequences of high similarity were retrieved from public databases.

RESULTS AND DISCUSSION

PCR, cloning and DNA sequencing has been completed for samples in disease categories 1 to 3 (Table 1).

Table 1. Prevalence of fungal species detected in disease categories 1 (no disease) to 3 (moderate disease) for all families combined.

Species	Disease Category			
	1	2	3	4
<i>Allantophomopsis</i> sp	2 ¹	2	0	na ²
<i>Catenulostroma</i> sp	3	3	3	na
<i>Cyclaneusma</i> sp	4	5	2	na
<i>Mycosphaerella</i> sp 1	8	6	4	na
<i>Mycosphaerella</i> sp 2	8	6	5	na
<i>Mycosphaerella pini</i>	5	6	2	na
<i>Lophodermium pinastri</i>	0	1	4	na
<i>Phoma</i> sp	1	2	1	na
<i>Tumularia</i> sp	5	3	0	na

¹the number of samples out of 9 in which a species was detected

²Data to be presented on poster.

In this study *Mycosphaerella* species 1 and 2 were common to all three disease categories. *Mycosphaerella pini* was common to classes 1 and 2 but less frequently detected in class 3. *Cyclaneusma* sp. was not clearly correlated with disease incidence in the three disease categories analysed. *Lophodermium pinastri* was found more frequently in needles from trees with a higher disease severity.

From the data so far collated, no clear association of any fungal species with disease incidence is evident. Further data analysis will be needed to study the correlation of fungal species with the host genetics.

ACKNOWLEDGEMENTS

Australian Research Council, Forestry Tasmania, Rayonier, Taswood Growers, Norske-Skog, Forests NSW, Hoskings Ltd. New Zealand. Istiana Prihatini is the recipient of a John Allwright Fellowship, ACIAR.

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7 A new report on *Pseudomonas syringae* pv. *mori* causal agent of bacterial blight of mulberries in Australia

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INTRODUCTION

Pseudomonas syringae pv. *mori* (Boyer & Lambert) Young *et al.* causes a leaf spot and blight of young shoots on mulberry. It has been a common disease of mulberry worldwide. Symptoms appear as small water-soaked leaf spots, turning brown or black, sometimes with a yellow halo. Spots on the midribs and vines are sunken. Infected leaves are often become distorted and bacterial ooze may be extruding from lenticels. Young shoots may show rapid necrosis and cankers (Fig. 1). The disease has been reported on mulberry (1) however, there is no official record of *P. syringae* pv. *mori* in Australia.

MATERIALS AND METHODS

Leaf spots and blight of young shoots were observed on mulberry trees in east of Perth in the summer of 2008 (Fig.1). Samples of leaf and infected branches were collected. Isolations were made from lesions on the leaf and stem tissues. Isolates with positive hypersensitivity on tobacco (*Nicotiana glutinosa*) were used for further tests. The bacterial isolates were identified based on biochemical tests (2) and using the Biolog identification system based on the carbon utilisation microplate assay (Biolog MicroLog 4.0 System, Biolog Inc., Hayward, CA).

Pathogenicity tests. To confirm identification of the bacterial strains, pathogenicity tests with two isolates were performed on immature lemon, pear fruits, young bean pods and tomato seedlings (3). Isolates were grown on sucrose peptone agar (SPA) for 24 h and then suspended in sterile water and diluted to a concentration of 10^6 CFU/ml. Fruits and bean pods were surface sterilised with alcohol, washed with sterile water and inoculated by placing drops of an aqueous bacterial suspension on the surface and pricked through the drops using sterile needles. Controls were inoculated with sterile water. After inoculation, fruits and bean pods were incubated in the moist trays at 25°C for 7 days. Four-week-old healthy tomato plants were inoculated using the same bacterial inocula, controls and techniques. Plants were placed under mist for 48 h and then moved to the growthroom chamber at $22 \pm 1^\circ\text{C}$. Disease symptoms were checked 7 days post-inoculation.

To test pathogenicity of the same *P. syringae* pv. *mori* isolates on *Malus alba*, young shoots and detached leaves were inoculated by placing drops of bacterial suspension (10^6 cfu/ml) on freshly wounded shoot and midrib tissues. Controls were inoculated in the same way using sterile water and then incubated in moist trays at 25°C.

RESULTS

A bacterial blight was found on mulberries in east of Perth in the summer of 2008. White-coloured and fluorescent bacterial colonies were consistently isolated from the leaf and stem tissues. Isolates were gram negative, fluorescent on King's medium B, oxidase negative, catalase positive, potato soft rot negative, arginine dihydrolase negative and tobacco HR positive. The representative isolates were tested using the biolog system and were identified as *P. syringae* pv. *mori* with a probability range of 96 to 100%.

The isolates caused necrosis of the shoots and tissue along the midribs of the mulberry leaves 7 days after inoculation (Fig.2). The isolates also caused water soaked lesions on the bean pods, pear and lemon fruits, although they did not produce disease symptoms. In leaves of tomato inoculated by pricking, chlorosis areas were seen 7 days after inoculation. Koch's postulates were fulfilled and reisolated bacterial colonies were identified as *P. syringae* pv. *mori*. Culture of *P. syringae* pv. *mori* has been deposited in the WA culture collection as WAC 13254.

To our knowledge, this is the first official report of *P. syringae* pv. *mori* on mulberry in Australia.

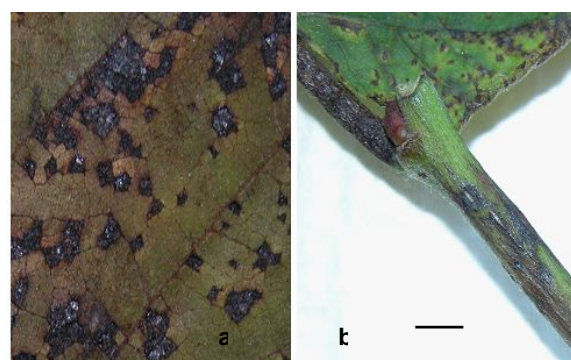


Figure 1. Disease symptoms; leaf spots (a) and blight of a young shoot (b). Bars = 1cm.

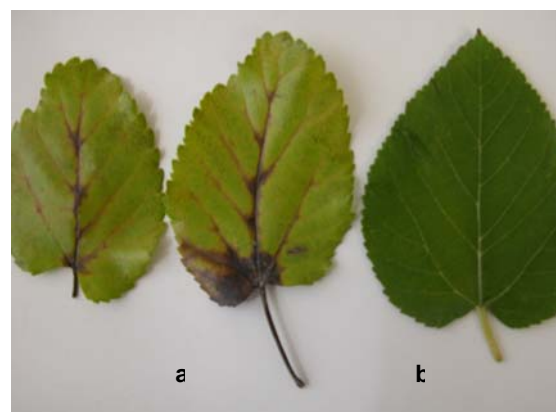


Figure 2. Pathogenicity test; Mulberry leaves showing necrosis symptoms (a), in comparison with a healthy leaf (b)

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47 Through chain assessment and integrated management of brown rot risks in stonefruit

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INTRODUCTION

Brown rot caused by *Monilinia fructicola* (G. Wint.) Honey is the major disease challenge for stonefruit growers and supply chain businesses in Australia. Crop losses are attributed to blossom blight and fruit rots, and occur despite fungicidal sprays applied by growers. Infection can be controlled with well-timed, effective fungicides (1); however, growers lack access to site-specific weather data and disease models to support control.

M. fructicola may infect during flowering, fruit development and after harvest, thus a through chain approach to disease management is required. There are many key management strategies including reducing inoculum potential, predicting infections, optimal timing of protectant and curative fungicides, understanding changes in host tissue susceptibility, controlling pests which vector the disease or assist infection and understanding the potential for postharvest disease. In this paper, we summarise our research in these areas and discuss the potential for their integration into a brown rot management strategy.

MATERIALS AND METHODS

Field sites. Trials were established in commercial orchards in the main Victorian stonefruit districts: 4 sites in 06/7, 7 in 07/8 and 10 in 08/9. Each site had 6 plots of 10 trees. A weather station was placed in the centre of the 4th plot at each site. These recorded day length, rainfall, leaf wetness inside and outside the canopy, RH and air temperature.

Inoculum for primary infection. In the first and second seasons, sites were surveyed during bloom for the presence of dried or mummified fruit in the trees and on the ground. Samples of these were collected (up to 20/plot) and moist incubated to detect *M. fructicola*.

Weather based prediction of infection risk. A weather-driven infection risk model (G Tate pers. comm.) was evaluated using data collected by the weather stations over 3 seasons. In the first season, surface wetness duration, a critical factor for infection risk was compared inside and outside tree canopies.

Effectiveness of fungicide programs. The predicted occurrence and severity of infection periods were examined in relation to fungicides applied by growers and brown rot incidence after harvest. Over the three seasons, growers made incremental changes towards spraying in response to predicted infection periods. The success of this was evaluated.

Influence of Carpophilus beetle populations on infection risk. At two sites, canning peach (var T204) blocks were treated with the carpophilus attract and kill system. Beetle populations and postharvest brown rot incidences were compared against untreated blocks.

Phenological influence on infection risk. In the 08/09 season, peach fruit (vars Golden Queen and T204) were spray inoculated at early shuck fall, post pit hardening and 1–2 weeks prior to harvest. Brown rot development was monitored during the growing season and postharvest.

A postharvest predictor of rot risk. At each site 20 fruit per plot were harvested at commercial maturity and moist incubated at 21°C for 7 and 12 days to establish the level of latent infections leading to rots.

RESULTS AND DISCUSSION

The abundance of mummified fruit infected with *M. fructicola* did not exclusively explain the incidence of postharvest rot (2). Tate's infection model was convenient for identifying periods of weather conducive to infection. However to make best use of the model it is necessary to understand a) the susceptibility of the crop at different phenological stages and b) the inoculum potential. In the first season of trials, inoculation of developing flowers and fruit at different growth stages did not reveal differences in tissue susceptibility and therefore, more comprehensive trials are planned.

There was a strong positive relationship between the number of moderate and severe infection risk events in the two weeks before harvest and the postharvest rot incidence. Well timed fungicides during this period appeared to have suppressed infections.

Flat plate sensors outside tree canopies generally recorded longer wetness intervals than sensors inside canopies, for both rain and dew events. This agrees with Henshall et al. (3) who showed this was the case in vineyards. Therefore wetness duration measured outside tree canopies will estimate greater disease risks (2).

Controlling carpophilus significantly reduced postharvest rot incidence and more work is required to determine how this fits into a disease control strategy.

Moist incubating samples of fruit collected a few days before commercial harvest can be used to estimate the risk of rots developing during storage, transport and marketing. Thus packers and distributors can appropriately treat and market high risk fruit into short supply chains, minimising wastage.

ACKNOWLEDGEMENTS

This project is supported by the Victorian Government, Summerfruit Australia, the Canned Fruit Industry Council and Horticulture Australia.

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48 Effects of temperature on mixed bunch rot infections of grapes

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INTRODUCTION

Although bunch rot of grapes is frequently associated with *Botrytis cinerea* or grey mould, this pathogen can be absent from bunch-rot affected vineyards under some climatic conditions. Of note is the occurrence of Ripe Rot and Bitter Rot caused by *Colletotrichum acutatum* and *Greeneria uvicola* respectively, in sub-tropical vineyards that experience warm and wet conditions close to harvest. In Australia, Ripe Rot and Bitter Rot have been recorded in coastal regions such as the Hunter Valley (NSW), Kingaroy (QLD) and Carnarvon (WA).

Previous studies have revealed that *C. acutatum* and *G. uvicola* were the predominant bunch rot pathogens isolated from berries collected at different phenological stages in the Hastings Valley (mid north coast NSW) (1), whereas isolation of *B. cinerea* was infrequent. Both *C. acutatum* and *G. uvicola* can occur concurrently in the one vineyard and even on the one berry. In an attempt to explore factors leading to the absence of *B. cinerea* from some vineyards, we investigated the ability of *C. acutatum*, *G. uvicola* and *B. cinerea* to co-infect berries at either 20°C or 27°C.

MATERIALS AND METHODS

Detached, disease-free Cabernet Sauvignon berries (22.4° Brix) were surface sterilised, rinsed in sterile water and placed into 24 well microtitre plates. Berries were inoculated either singularly or with combinations of *B. cinerea*, *C. acutatum* and *G. uvicola* (10 µL droplet on the distal apex of the berry, 10⁶ spores/mL) using three replicates per isolate with 24 berries per replicate. Berries were incubated for five days at either 20°C or 27°C in the dark at 100% RH. Berry colonisation was assessed by plating grape berries onto potato dextrose agar. Results were expressed as the mean percentage of berries infected.

RESULTS AND DISCUSSION

Grape berries were susceptible to infection by all three of the bunch rot pathogens examined. A higher percentage of berries were infected by *B. cinerea* at 20°C than at 27°C, while *G. uvicola* infection was favoured at 27°C. There was little difference in the infection of grape berries by *C. acutatum* at either temperature (Table 1).

The colonisation of grape berries by *B. cinerea* was not affected by co-inoculation with either *C. acutatum* or *G. uvicola* at 20°C but was reduced at 27°C. Conversely the growth of *C. acutatum* and *G. uvicola* was reduced by co-inoculation with *B. cinerea* at 20°C and not at 27°C. *G. uvicola* failed to colonise any berries at 20°C when co-inoculated with *B. cinerea*. *C. acutatum* also reduced berry infection by *G. uvicola* when co-inoculated at either temperature. *G. uvicola* had no effect on berry colonisation by *C. acutatum* at either of the temperatures examined.

These observations were further confirmed by inoculating grape berries with all three bunch rot pathogens at the same time. At 20°C *B. cinerea* was the pre-dominant pathogen while at 27°C *C. acutatum* predominated.

Table 1. Effect of co-inoculating *Colletotrichum acutatum* (Ca), *Greeneria uvicola* (Gu) and *Botrytis cinerea* (Bc) on disease expression in *Vitis*

vinifera cv. Cabernet Sauvignon berries (22.4° Brix). Control berries were water inoculated.

Treatment		% berries infected with		
Inoculum	Temp °C	<i>B. cinerea</i>	<i>C. acutatum</i>	<i>G. uvicola</i>
Control	20	0	0	0
	27	0	0	0
Bc	20	92	-	-
	27	65	-	-
Ca	20	-	96	-
	27	-	100	-
Gu	20	-	-	57
	27	-	-	99
Bc + Ca	20	82	65	-
	27	29	97	-
Bc + Gu	20	95	-	0
	27	46	-	99
Ca + Gu	20	-	95	12
	27	-	96	55
Bc + Ca ++ Gu	20	88	28	0
	27	45	96	45

B. cinerea is frequently associated with bunch rot of grapes in cool climates. Our results support earlier observations on the optimum climatic conditions for grey mould development (2). The sub-tropical climatic conditions of regions experiencing Ripe Rot and Bitter Rot are likely to pre-dispose berries to these diseases. Our additional observations (unpublished data) on the relative growth rates of the three pathogens on PDA, at a range of temperatures, support this hypothesis and may partially explain the absence of grey mould in sub-tropical vineyards.

ACKNOWLEDGEMENTS

This work was supported by the Winegrowing Futures Program, a joint initiative of the Grape and Wine Research and Development Corporation and the National Wine and Grape Industry Centre.

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72 Genetic diversity of Iranian *Fusarium oxysporum* f. sp. *ciceris* by RAPD molecular markers

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INTRODUCTION

Fusarium oxysporum Schlecht, Emend (Snyder & Hansen) is one of the most important soilborn plant pathogens with a worldwide distribution. One of the most important crops in Iran is the Iranian chickpea (*Cicer arietinum* L.) with the annual production of 260,000 tons from 755,000 hectares. *Fusarium* wilt of chickpea is a devastating disease in chickpeas growing in different regions of Iran. This fungal disease is caused by *Fusarium oxysporum* f.sp. *ciceri*. Characteristic symptoms of disease are leaves necrosis, yellowing, vascular wilt and damping-off (2).

Iran is the world's fourth important chickpea producing countries and, this pathogen can reduce yield about 15%, so an investigation of genetic diversity of this pathogen in the regions seems to be of great significance.

MATERIALS AND METHODS

Thirty isolates of *Fusarium oxysporum* f.sp. *ciceri* with different geographical origins were chosen for genetic diversity studies. *In vitro* pathogenicity tests were performed using a root-dip assay, cluster analysis of the isolates classified into three categories of highly, moderate and weakly virulent groups. DNA extraction was performed using Readers & Borda method with few modifications (3). For RAPD analysis thirty random primers were screened and ten primers producing the highest number of scorable bands were selected for the final analysis (Table 1). 20 ng of genomic DNA from each isolate was amplified with the selected primers. Amplified DNA was cluster analysed using MVSP software and UPGMA method with jaccard coefficient.

Table 1. Sequence of primers used in this study.

primers	Sequences 5-3
1	CCG GCC TTA G
2	ACC GGG TTT C
3	GGG GGG ATC A
4	CCT GGC GGT A
5	CCT GTG CTT A
6	CCT GGG CTT G
7	CCT GGG GGT T
8	CCT GGG CTT C
9	CCT GGG CCT A
10	CCT GGG TTC C

RESULTS AND DISCUSSION

Results showed that there is a high genetic diversity among *F. oxysporum* isolates. Honnareddy & Dubey (2005) probed the genetic diversity of the aforementioned fungus utilising RAPD technique and isolates were classified into seven categories (1). Singh (2006) through the investigations performed on 30 isolates of *F. oxysporum* f.sp. *ciceri* collected from North India, observed little genetic variability and classified the isolates into three clusters (4).

Through our investigation of polymorphic bands, 15 bands were observed. Considering a 70% similarity on dendrogram diagram genotypes were classified into 8 clusters (figure 1). Our results of RAPD-PCR demonstrated the existence of polymorphism in the fungi populations, and a high genetic diversity was also observed among isolates under investigation. According to existence or non-existence of bands, the genotypes classification has not matched geographical localisation. With respect to the fact that there is no significant correlation between the geographical origin of isolates and polymorphic bands, the occurrence of such a condition could be the result of seed exchange between the farmers.

It seems that the more the polymorphic bands are the more is the possibility of recombination and genetic diversity in pathogens which is in turn due to their ability to mutate and anastomosis with other isolates. This will result in resistance break down against the pathogen in resistant cultivars. Due to the fact that resistant cultivars are used to control this disease, when genetic characteristic of the pathogen population changes continuously, we should prevent resistance break down by relentless reviewing of the genetic diversity on the one hand and searching for new resistant cultivars on the other hand.

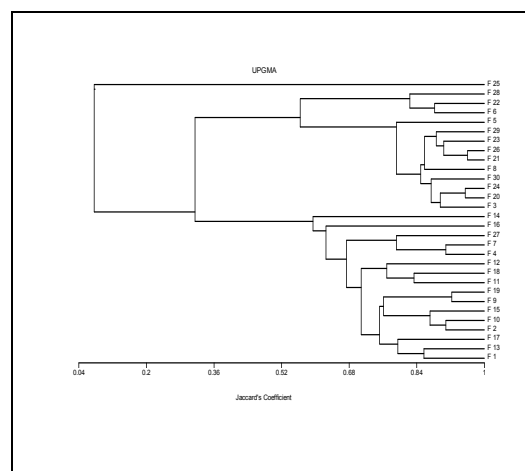


Figure 1. Dendrogram derived from RAPD analysis of *Fusarium oxysporum* f.sp. *ciceri* by UPGMA.

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49 Development of nationally endorsed diagnostic protocols for plant pests

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INTRODUCTION

In 2005, a new “committee on plant diagnostics and laboratory accreditation” was formed as a subcommittee of Plant Health Committee (PHC). Called the Subcommittee for Plant Health Diagnostic Standards (SPHDS), the primary goal was to “establish, implement and monitor professional and technical standards within plant health diagnostic laboratories through the development and maintenance of an accreditation system and national diagnostic standards”.

The Diagnostic Standards Working Group (DSWG) of SPHDS has developed a set of reference standards (1) to assist potential authors in developing diagnostic protocols for the detection and identification of plant pests, particularly the 253 organisms categorised as being of high importance under the Emergency Plant Pest Response Deed (2) and Industry Biosecurity plans. The standards are consistent with the international standard for diagnostic protocols for regulated pests. (3)

PROTOCOL DEVELOPMENT

National Diagnostic Protocols are defined as “A PHC endorsed Australian document containing detailed information about a specific plant pest or group of plant pests relevant to its diagnosis”. They are designed to assist diagnosticians in the identification of a specific pest and include data on the pest, its hosts and taxonomy, methods for detection and identification, acknowledgements, references and contacts for further information.

New protocols are often developed with the assistance of a scholarship to work in an overseas laboratory, as it is not always possible to bring positive controls into Australia. Information for many of the pests of concern is available on the Plant Biosecurity Toolbox (4), part of the Pest and Disease Image Library (PaDIL). Draft protocols are developed by authors utilising information from the Plant Biosecurity Toolbox and then submitted to SPHDS for assessment.

ASSESSMENT PROCESS

When protocols are submitted to SPHDS, the DSWG form an Assessment Panel, comprising members of DSWG and other “experts” as deemed necessary by SPHDS. The protocols are assessed using the criteria outlined in Reference Standard (RS) No. 3 (1). In collaboration with the author, the Assessment Panel facilitates verification and peer review of the protocol according to RS No. 4 (1). Verification is undertaken by an independent laboratory with the aim of demonstrating whether the diagnostic procedures can be followed. Peer review is where an expert of the pest area reviews the accuracy and currency of the scientific information provided in the submitted diagnostic protocol, similar to a journal review. Once both Verification and Peer Review reports are received by SPHDS, the Assessment

Panel reconvenes and determines whether the protocol has been deemed acceptable, or more revision is required.

Once accepted, the Assessment Panel recommends to SPHDS that the completed protocol be submitted to PHC with a recommendation for endorsement as a National Diagnostic Standard.

ENDORSED NATIONAL DIAGNOSTIC PROTOCOLS

Protocols endorsed by PHC are placed as a version controlled document on the SPHDS website (5) to be used as part of a national response to emergency plant pest incidents for specific pest species. In some instances they may also be suitable for use in surveys to demonstrate evidence of absence to enable market access of Australian produce.

Currently there are two endorsed protocols:

- NP1—Apple Brown Rot (*Monilinia fructigena*)
- NP2—Plum Pox Virus

The protocols are reviewed every three years and if necessary are subject to rewriting and resubmission to SPHDS.

FUTURE WORK

DSWG is in the process of facilitating the verification and peer review of another 15 protocols. It is anticipated that at least 10 of these will be completed and endorsed by the end of 2009. With 253 important pests on the list to do, not counting other high risk regulated pests and others that may appear unexpectedly, there is still a lot of work ahead.

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96 Subcommittee on Plant Health Diagnostic Standards

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INTRODUCTION

In 2005, a new "committee on plant diagnostics and laboratory accreditation" was formed as a subcommittee of Plant Health Committee (PHC). Called the Subcommittee for Plant Health Diagnostic Standards (SPHDS), the primary goal was to "establish, implement and monitor professional and technical standards within plant health diagnostic laboratories through the development and maintenance of an accreditation system and national diagnostic protocols". This is all part of a push to facilitate activities that will enhance Australia's plant biosecurity.

WORK IN PROGRESS

Laboratory accreditation: Several different models were explored with accreditation to the international standard AS ISO/IEC 17025 (1) adopted as the way forward. Steps in development of a Field Application Document (FAD) included: draft FAD incorporating plant health diagnostic testing with the requirements for Veterinary Testing, independent FAD for the field of Plant Health Diagnostic Testing and, most recently, incorporation of plant health testing requirements into the Biological Testing FAD.

A revised edition of the Biological Testing FAD incorporating a Plant Health Diagnostic Testing Annex should appear on the NATA website shortly (5).

Diagnostic Protocols. The Diagnostic Standards Working Group (DSWG) of SPHDS has developed a set of reference standards (2) to assist potential authors in developing diagnostic protocols for the detection and identification of plant pests, particularly those categorised as being of high importance. These reference standards provide a standardised format for protocols and describe a process for assessment involving verification and peer review (2). Most, but not all, of the organisms on the list for protocol development come from the Emergency Plant Pest Response Deed (4) and from industry biosecurity planning processes.

Currently two National Diagnostic Protocols have been endorsed by Plant Health Committee and can be found on the SPHDS website (3).

Diagnostic Services. Diagnostic service capability and capacity in Australia is a critical issue. SPHDS is involved in multiple ways of highlighting the issues and developing strategies. These include:

- DAFF Training scholarships, administered by SPHDS, help to increase diagnostic capacity by sending diagnosticians overseas for training. There is an expectation that a draft diagnostic protocol would be an outcome of a scholarship. Scholarships have been awarded each year since 2004. Thirty-eight scholarships had been awarded by the end of 2008.

- National Diagnostic Strategy. PHC has charged SPHDS with developing a National Diagnostic Strategy for plant health. This is additional to the National Plant Health Strategy developed by Plant Health Australia.
- Training Workshops. One of SPHDS tasks is to prioritise and coordinate training in the diagnostic community.

FUTURE ACTIVITIES

Enhancing plant biosecurity for Australia is integral to successful plant health management. Having readily available, soundly based, diagnostic protocols for plant pests plays an important role. The magnitude of the task facing DSWG is illustrated by the over 230 plant pests listed in Table 4 of the National Plant Health Status Report(6) that require protocol development. Laboratory accreditation assures quality and integrity of the results produced and provides confidence to biosecurity administrators in managing biosecurity emergencies. Information on plant health laboratory accreditation and what it means for plant health diagnosticians will be prepared.

ACKNOWLEDGEMENTS

The guidance of NATA staff in preparation of the FAD documents is gratefully acknowledged.

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97 What is laboratory accreditation and what will it mean for me and my laboratory?

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INTRODUCTION

Accreditation is seen as a key plank in supporting the improvement of capability and capacity in Australian plant health diagnostic laboratories to respond to biosecurity emergencies and is a key part of the business of Subcommittee on Plant Health Diagnostic Standards (1).

There are two types of accreditation that could apply to plant health laboratories. The first of these examines the ability of a laboratory to secure and contain plant pests while undertaking diagnostic procedures and is administered by Australian Quarantine Inspection Service (AQIS). To this end a number of Quarantine Containment or QC Levels are recognised. This scheme is not discussed here.

The second type of accreditation is an internationally recognised system for Quality Assurance based on the international standard AS ISO/IEC 17025 (2) administered in Australia by National Association of Testing Authorities (NATA) (3). This paper details the development of this scheme and how it will affect you.

MATERIALS AND METHODS

Several plant health diagnostic testing laboratories are currently accredited to AS ISO/IEC 17025 under the Biological Testing Field Application Document (FAD). An additional Annex to this FAD has been developed to specifically cover plant health diagnostic laboratories.

To get to this stage, SPHDS explored several different models, with accreditation to the international standard AS ISO/IEC 17025 (2) adopted as the way forward. Steps in development of a FAD included: drafting plant health diagnostic testing requirements for incorporation with the Veterinary Testing FAD, drafting an independent FAD for the field of Plant Health Diagnostic Testing and, most recently, incorporation of plant health testing requirements into the Biological Testing FAD.

RESULTS

A revised edition of the Biological Testing FAD incorporating a Plant Health Diagnostic Testing Annex should appear on the NATA website shortly (3).

DISCUSSION

There are significant advantages in developing and operating according to an internationally recognised quality assurance system. It is recognised that there are also some disadvantages including the cost associated with developing and maintaining the system.

What will it mean to laboratories and individuals working in them? This will be discussed in the context of examples from laboratories already accredited.

ACKNOWLEDGEMENTS

The support of NATA, Plant Health Australia and Plant Health Committee, the parent body of SPHDS, is gratefully acknowledged; together with the input from staff of already accredited laboratories who have given examples of what it means to be accredited from their perspective.

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26 In vitro fungicide sensitivity of *Botryosphaeriaceae* species associated with 'bot canker' of grapevine

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INTRODUCTION

Botryosphaeriaceae species are commonly associated with the grapevine trunk disease, 'Bot canker'. This disease is a serious threat to the productivity and longevity of vineyards in Australia and begins when pruning wounds are infected by these fungi. Consequently, damage to the vascular system of the vine limits vegetative growth and reduces yield. Control strategies emphasise the protection of wounds against infection, and a number of chemicals have been screened *in vitro* for inhibition of the *Botryosphaeriaceae* (1). To date, there are still no fungicides registered for the management of 'Bot canker' in Australia. Based on a previous study (1), 10 fungicides were selected and further evaluated for their activity on four additional *Botryosphaeriaceae* species recently isolated from diseased grapevines in New South Wales and South Australia. The aims of this research were to determine the sensitivity of these species to chemical fungicides and to identify potential agents for management of the *Botryosphaeriaceae*.

MATERIALS AND METHODS

The active ingredients of 10 fungicides were evaluated *in-vitro* for mycelial inhibition of five isolates each of *Diplodia mutila*, *Neofusicoccum australe*, *Dothiorella viticola*, and *Dothiorella iberica* (Table 1). Agar plugs (5 mm diameter) from the margins of actively growing four-day-old fungal cultures were transferred to fungicide-amended-agar plates. Fungicides were dissolved in acetone (<0.1%), added to molten agar and assessed at concentrations between 0 to 10 mg/L. Three replicate plates per isolate × fungicide × concentration combination were inoculated and incubated at 25°C for two to three days. Mean colony diameter was determined for each plate and the percentage growth inhibition relative to the control calculated. Percentage inhibition data were fitted over fungicide concentrations for each isolate and fungicide using a log-probit regression. Data were normalised by logarithmic transformation and EC₅₀ (concentration of fungicide at which 50% of mycelial growth is inhibited) values calculated. Differences in treatment effects were determined by an analysis of variance (ANOVA) and means separated using Duncan's multiple range test (DMRT; $P = 0.05$). Means were back-transformed to the original scale. The experiment was conducted twice. A Bartlett's test for homogeneity of variance between trials was not significant ($P=0.168$). Hence, data from the 2 trials were combined.

RESULTS AND DISCUSSION

Fungicides differed significantly in their ability to inhibit mycelial growth, with some interactions apparent between specific isolates and fungicides ($P = 0.05$). Fluazinam, fludioxonil, carbendazim, tebuconazole and flusilazole were the most effective with EC₅₀ values of 0.01, 0.01, 0.04, 0.07 and 0.08 mg/L, respectively (Figure 1), while penconazole and procymidone were intermediately effective with EC₅₀ values of 0.23 and 0.29, respectively.

Iprodione, myclobutanil and pyraclostrobin were the least effective with the results of this study corroborating those of previous studies on four other *Botryosphaeriaceae* species (1). Field evaluation of these chemicals for protection of pruning wounds is currently under way. Combined with field trials these

studies provide valuable information for future management of fungi associated with 'Bot canker'.

Table 1. Fungicides tested for mycelial inhibition of *Botryosphaeriaceae* species.

Fungicide	Chemical group
Carbendazim (Carb.) ^a	A
Fluazinam (Flua.) ^a	Y
Fludioxonil (Flud.) ^a	L
Flusilazole (Flus.) ^a	C
Iprodione (Ipro.) ^b	B
Myclobutanil (Mycl.) ^b	C
Penconazole (Penc.) ^b	C
Procymidone (Proc.) ^b	B
Pyraclostrobin (Pyra.) ^b	K
Tebuconazole (Tebu.) ^b	C

^aFungicide concentrations—0, 0.005, 0.0075, 0.01, 0.05, 0.1 mg/L.

^bFungicide concentrations—0, 0.5, 1, 2.5, 5.0, 10.0 mg/L.

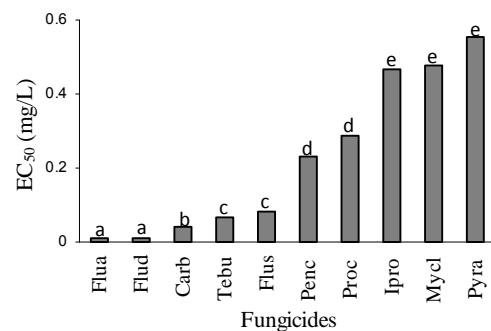


Figure 1. Sensitivity of *Botryosphaeriaceae* species to various fungicides. Fungicides followed by the same letter are not significantly different ($P = 0.05$).

ACKNOWLEDGEMENTS

This work was supported by the Winegrowing Futures Program, a joint initiative of the Grape and Wine Research and Development Corporation and the National Wine and Grape Industry Centre.

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50 The impact and diversity of *Mycosphaerella* leaf disease isolated from *Eucalyptus globulus* in western australia

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INTRODUCTION

The most serious foliar disease of eucalypt plantations in WA is *Mycosphaerella* leaf disease (MLD) (1). Since the commencement of the plantation industry, several fungal species contributing to MLD, previously known only in eastern Australia or overseas, have been reported on *E. globulus* in WA. Initially only three species were identified (2). More recently, five new records from WA (*M. aurantia*, *M. ellipsoidea*, *M. mexicana* and *M. fori*) have been identified that have not been recorded elsewhere in Australia (1, 3). Currently, 13 species of *Mycosphaerella* have been recorded in WA from *Eucalyptus* (3). Re-examination of cultures adds six new species that have yet to be described from *E. globulus* in WA. The impact of MLD on growth of *E. globulus* plantations in WA was examined in a chemical exclusion trial at two plantations in the Albany region.

MATERIALS AND METHODS

Plantations were surveyed over the period 2001–2006 for MLD and associated pathogens. Lesions from infected leaves were soaked 20–60 mins before being blotted dry and placed on the lid of a Petri dish. Single spore isolations were made according to (1). Thirty spore measurements of each species were made at 1000x magnification. The internal transcribed spacer region (ITS) was sequenced to confirm the identity of each species.

To examine the effect of controlling MLD, an experiment was conducted on two (A and B) one-year-old commercial *E. globulus* plantations and consisted of four spray treatments (fungicide, insecticide, fungicide plus insecticide and non-treated controls), replicated 5 times with 50 trees per replicate. This regime was designed to determine whether controlling pest and fungal diseases for 2–3 yrs increases above-ground biomass at 2 and 5 yrs. The systemic fungicide benomyl (Benlate[®], DU PONT Australia Ltd), and chlorothalonil (Bravo[®] 500 DU PONT Australia Ltd) or chlorothalonil/ethylene glycol (Rover[®] 500 Flowable, NUFARM Australia Ltd), were used alternately to ensure fungicide resistance would not occur. Alphacypermethrin (Dominex[®] 100, Crop Care Australasia Pty Ltd) was applied regularly to control insects. Tree height and stem diameter were measured prior to the experiment (1 yr) and twice thereafter (3 and 5 yrs). Volume was calculated using ITC's standard equation and standardised for statistical analysis.

RESULTS AND DISCUSSION

The current study documents an increase in the number of *Mycosphaerella* species associated with *E. globulus* plantations in WA from 13 to 19. There are a number of important implications that arise from these detections including the potential impact on plantations in WA; biosecurity implications of the origin and spread of eucalypt diseases; and the ecological function of the diverse *Mycosphaerella* assemblage that is associated with *Eucalyptus* forests and plantations.

While site differences had the greatest effect on standardised tree volumes of blue gums between 2002 and 2004 in the chemical trials, there were also significant treatment effects. The application of fungicides and insecticide increased wood

volumes by 2.9%–13.5%. The critical question from a management viewpoint is whether the demonstrated increases in standardised tree volume were sufficient to warrant the cost of fungicide and insecticide treatments of the trees. Significantly, the plantations experienced a very low incidence of disease and pest attack during the trial period. Even so, the results clearly showed a significant difference between treatment types and disease outcome. This suggests that the use of chemical treatments may be useful in controlling disease outbreaks. However, the treatments most likely would have to be ongoing.

Although MLD in WA occurs at relatively low levels compared to other states in Australia, the fact that the diversity of species has not yet stabilised is a concern. The number of new species isolated is steadily increasing, however, our knowledge of the biology and epidemiology of these organisms remains largely unchanged. *M. cryptica* and *M. nubilosa* are the two most important species found in WA. However, with the increasing number of species being recorded in WA, the chance of finding other significant pathogenic species is high. The industry should not remain complacent, and a concerted effort should be made to remain vigilant. Although field diagnosis remains problematic, monitoring plots across the state of varying ages should be established and outbreaks investigated in detail. The efficacy of Forest Stewardship Council accredited fungicides on MLD should be investigated in case of severe outbreaks in the future.

ACKNOWLEDGEMENTS

This work was part of an ARC Linkage (LP0219585). ITC Ltd was the industry partner and their financial and in kind support is gratefully acknowledged.

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8 Efficacy of pre-seeding fungicides for control of barley loose smut

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INTRODUCTION

In recent years, loose smut caused by *Ustilago nuda* (Jensen) Kellerman & Swingle has been seen widely and caused yield losses in barley (*Hordeum vulgare* L.) crops along the south coast of Western Australia (WA). Barley is an increasingly important crop in this region, which has an environment suited to spread and development of this disease. All the major malting barley varieties grown in WA, including Baudin which is widely adopted in this region, are susceptible to loose smut. Increased barley cropping area in disease favourable environments, widespread utilisation of susceptible varieties and changes in seeding fungicide usage towards fertiliser applied fungicide to manage diseases such as powdery mildew has raised concern amongst WA south coast barley producers over the re-emergence of loose smut. The aim of the current study was to evaluate the efficacy of some pre-seeding fungicides available in the Australian market for loose smut control.

MATERIALS AND METHODS

At three geographically separate locations, field trials were carried out using naturally infected seed of Baudin barley. The fungicide rates used are shown in Table 1. All trials were randomised block designs with four replicates. Assessments were made at each site of tiller counts, loose smut incidence and grain yield.

RESULTS AND DISCUSSION

Significant reductions in disease transmission were evident from most of the treatments, however none of the tested products completely eradicated transmission of loose smut (Table 1). The level of disease transmission and the relative efficacy of some fungicide products varied between experimental sites, as previously reported by Loughman *et al.* (1). *Triadimefon* (Baytan), *triticonazole* (Real) and *tebuconazole* (Raxil) based products significantly reduced loose smut at all sites. At the rates used in these experiments, *fluquinconazole* (Jockey) and *difenoconazole* (Dividend) gave variable responses, being effective at only some of the experimental sites. *Triadimefon* (Triad IF) applied to fertiliser and banded with seed was ineffective at all sites. Yield responses to fungicide applications were noted at Gibson, ranging from 4.6 to 5.1 t/ha and Mt Barker 3.9 to 4.4 t/ha respectively. Increased yield at these two sites does not appear related to loose smut control but was possibly due to reductions in foliar diseases such as powder mildew. The yield at Avondale ranging from 2.6 to 2.8 t/ha and treatment difference were not significant (data not shown).

The current study supports industry observations that complete control of loose smut infection by fungicide application is unlikely. Fungicide seed treatments vary in efficacy of loose smut control and the efficacy of individual products can vary significantly between locations. However, current study does show that application of recommended rates of registered fungicide seed treatment will reduce loose smut infection such that regular use should diminish the occurrence of loose smut. Control of loose smut remains difficult where choice of fungicide and application method is determined by need to manage diseases other than loose smut, such as in-furrow fungicide for powdery mildew or Dividend seed dressing for root disease control. In these situations it will be required to combine disease

management strategies to combat loose smut and other diseases. Increased availability of varietal resistance to control loose smut would assist in the management of the disease and could simplify control and management options where loose smut has traditionally occurred with other diseases of barley.

Table 1. Effect of seed dressing and in-furrow fungicides on loose smut incidence in Baudin barley at Avondale, Mt Barker and Gibson, 2005.

Treatments [*]	Incidence infected heads (% heads infected) ^{**}		
	Avondale	Mt Barker	Gibson
Untreated	0.39 (3.5) ^{a†}	0.15 (2.2) ^a	0.07 (1.5) ^a
Dividend ^{LO}	0.30 (3.0) ^{ab}	0.08 (1.5) ^a	0.10 (1.8) ^a
Jockey ^{LO}	0.13 (1.9) ^c	0.08 (1.5) ^a	0.06 (1.4) ^a
Baytan ^{LO}	0.15 (2.2) ^{bc}	0.00 (0.0) ^b	0.01 (0.4) ^b
Real ^{LO}	0.10 (1.7) ^c	0.01 (0.2) ^b	0.00 (0.2) ^b
Raxil ^L	0.07 (1.4) ^c	0.01 (0.4) ^b	0.02 (0.6) ^b
Triad IF ^O	0.42 (3.7) ^a	0.17 (2.2) ^a	0.07 (1.3) ^a
<i>p</i>	<0.001	<0.001	0.046
lsd	(0.9)	(0.8)	(1.0)

Note:

^{*}Treatments: Dividend @ 100–130 mL/100 kg seed; Jockey @ 450 mL/100kg seed; Baytan @ 150 mL/100kg seed; Real @ 150 mL/100kg seed; Raxil @ 100 mL/100kg seed; Triadimefon 500WP @ 200g/ha in-furrow

^{**}Means of raw data presented, angular transformation used for statistical analysis presented in parenthesis.

[†]Means followed by the same letter in the same column are not significantly different at the $p = 0.05$ level.

^LFungicides registered for loose smut

^OFungicides registered for other barley diseases

ACKNOWLEDGEMENTS

We would like to thank the Department of Agriculture and Food Research Support Units at Avondale, Mt. Barker and Esperance for the field operations. Grain Research and Development Corporation (GRDC) for funding the research (DAW00106).

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73 The cause of the barley leaf rust in Western Australia is a typical *Puccinia hordei*

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INTRODUCTION

Leaf rust of barley caused by *Puccinia hordei* exists in most areas in which barley is grown. The Australian populations of this rust fungus, especially those of Western Australia (WA), are isolated from mainland Asian and South African populations by thousands of kilometers.

It is of great interest to compare characters (other than pathotypes) of the WA rust population to the Israeli, because Israel is located in the centre of the cultivated barley origin. The wild ancestor—*Hordeum spontaneum* and the alternate host—*Ornithogalum* spp. still exist in the area. The sexual stage of barley leaf rust is found annually all over the northern part of Israel.

MATERIALS AND METHODS

Teliospore germination and inoculation of alternate aecial host.

Telia originated on cultivated barley fields from the southern region of WA and from wild barley (*H. spontaneum*) in central Israel, were used for inducing teliospore germination and inoculate *O. eigii* plants in the greenhouse (1).

DNA content of pycniospore nuclei. Pycniospores were harvested from pycnial clusters on *O. eigii*. Stained for 2h with propidium iodide in TRIS-HCl buffer containing RNase and TritonX-100. Relative DNA content was determined by flow cytometer (FACS). Fluorescence intensity was measured (1).

Teliospores morphology. Teliospores were mounted in 50% glycerol on glass slides. Images were obtained with a digital camera. Spore dimensions were analysed using image analysis software.

Staining of Substomatal Vesicles (SSV). Segments taken from inoculated barley leaves were microwaved in 0.03% trypan-blue in lactophenol-ethanol for 60 s, cleared in chloral hydrate, and mounted in lactophenol for microscope examination. Images were taken with digital camera (1).

RESULTS AND DISCUSSION

Teliospore germination and inoculation of the alternate host.

Figure 1 shows the pycnial and aecial clusters of *P. hordei* on *O. eigii*. The WA isolates proved to be infectable (after inducing teliospore germination) to the alternate host—*O. eigii*, pycnial and aecial clusters were found (Fig. 1B). The aeciospores were infectable on barley seedlings, giving rise to uredinial sori (greenhouse experiments).

Crosses between WA and Israeli isolates. Crosses of WA isolates and Israeli isolates in both directions of nectar transfer could be achieved, and gave rise to aecial clusters (Fig. 1A). Analysis of differential host range of the hybrids in comparison to their parents is not of the scope of this abstract (2).

Nuclear DNA content spore morphology and SSV. Comparison of the isolates from WA and Israeli by DNA content, spore morphology and SSV show very close similarities (Figs. 2, 3, 4).



Figure 1. Pycnial and aecial clusters of *P. hordei* on *O. eigii*, after artificial inoculation in the greenhouse (21±2 °C). **A).** Single aecial cluster (at the center of it a few pycnia may be seen). Inoculation with Israeli isolate #1930, fertilisation of the pycnial cluster with pycniospores of WA isolate #22507. **B).** Pycnia and aecia can be seen along the rachis and fruit after inoculation with WA isolate #22507.

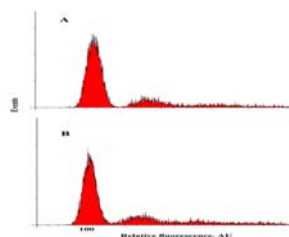


Figure 2. Histograms showing number of nuclei of given fluorescence intensities obtained by flow cytometry for propidium iodide-stained pycniospores of the leaf rust isolates of **A).** Israeli #1946 **B).** WA #22507. About 10000 pycniospores were measured in each FACS run. The similar position of the two histograms on the X axis points out a similar content of nuclear DNA in the pycniospores of both isolates.



Figure 3. Teliospores of *P. hordei* isolates from **A).** Israeli #1963 and **B).** WA #22507 and dimensions. Bar = 20 µm for both figures. Dimensions: area (µm²), length (µm) and width (µm); **A):** 774±101, 47±5 and 21±2; **B):** 776±96, 45±5 and 22±2.

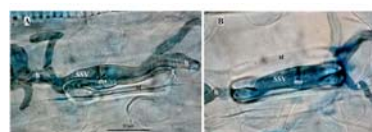


Figure 4. Uredinial substomatal vesicles (SSV) formed in barley cultivar L-94 by: **A).** Israeli isolate #1930, and **B).** WA isolate #22507. SSV—substomatal vesicle; ms—median septum; h—hyphae; st—invaded stoma. The SSV of the two isolates are very similar in shape and in dimension. Bar = 20µm.

Our results give rise to the opinion that despite geographic isolation, the WA population of *P. hordei* is taxonomically similar to isolates from outside Australia. It may have either reached Australia quite recently (in an evolutionary sense a few hundred years ago) with a very low rate of changes or there is some way of connection (winds, or another way) of the WA population and international populations.

ACKNOWLEDGEMENTS

Authors wished to thank Drs. Robert Park, University of Sydney and Robert Loughman, Department of Agriculture and Food Western Australia for initial reviewing of the abstract.

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27 The value of combined use of genetic resistance and fungicide application for management of stripe rust

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INTRODUCTION

Stripe rust (yellow rust) of wheat (*Triticum aestivum* L.) caused by *Puccinia striiformis* f. sp. *tritici* was first detected in Western Australia (WA) in 2002. Regional outbreaks have caused considerable yield losses, particularly in susceptible wheat varieties. Wheat varieties grown in WA range from susceptible to resistant, with many exhibiting varying degrees of partial resistance. The most effective use of fungicides in combination with these varying levels of resistance is poorly understood. The aim of the present study was to determine how varieties with different levels of rust resistance respond to fungicide for the control of stripe rust.

MATERIALS AND METHODS

Varieties with a range of stripe rust resistance, EGA Bonnie Rock (S-VS), Carnamah (MS-S), Wyalkatchem (MS), Janz (MR-MS) and GBA Ruby (R) were tested in combination with 3 fungicide treatments, being either nil, partial or full fungicide control, during 2007 and 2008. Trial design was split plot with four replications. Full control consisted of *tebuconazole* (Folicur 430SC) @ 290 mL/ha applied at early stem elongation (Z31), flag leaf emergence (Z39/40), ear emergence (Z55) and late flowering (Z68) to provide maximum disease protection and yield potential. Partial fungicide control consisted of a single application at ear emergence (Z55) in 2007 or two applications commencing with the first sign of the stripe rust (Z32) and again at ear emergence (Z55) in 2008. In 2007, the trial was sown on 5 July, adjacent to susceptible wheat (cv. Harrismith) inoculated twice on 30 July and 23 August to generate inoculum. In 2008 the trial was sown 20 June adjacent to susceptible wheat (cv. Westonia) that was inoculated with stripe rust on 24 July.

RESULTS AND DISCUSSION

In both years, the stripe rust was evident between stem extension and flag leaf emergence. In 2007, the stripe rust severity ranged from 4 to 96% in untreated control plots of the five varieties whereas in 2008, it varied from 6 to 93% (Figure 1a). GBA Ruby had no response to fungicide for stripe rust control. Application of fungicides either as single, double or multiple sprays reduced the stripe rust levels in all other wheat varieties, in both years. Under these experimental circumstances, where the varieties were subject to continuous high disease pressure from nearby infected susceptible wheat, partial fungicide control was less effective than full control with multiple fungicide sprays in Carnamah, EGA Bonnie Rock, Janz, and Wyalkatchem.

Over two years, extreme yield loss (87–94%) was observed in EGA Bonnie Rock (Figure 1b). In Janz and Wyalkatchem, partial resistance reduced the impact of stripe rust however yield losses of 27–54% were still observed. Partial control combined with partial resistance reduced yield losses to 17–30%, depending on variety. Application of fungicides significantly increased the yield and hectolitre weight in all the varieties tested except for GBA Ruby.

In 2007, screenings varied from 3.3 to 18.4% among the untreated varieties whereas in 2008, it was 0.8 to 7.9% (data not shown). EGA Bonnie Rock had higher screenings in both years.

These experiments demonstrate the effect of single major gene resistance. Under high disease pressure, stripe rust infection in GBA Ruby was very low and maximum yield was achieved without fungicide protection. However, Australian and international experience is that single major gene resistance to stripe rust, though highly effective, is not durable while multiple gene resistance is more robust. GBA Ruby carries Yr27, which is currently fully effective in WA but recent reports indicate development of Yr27 virulence in the stripe rust population in eastern Australia.

Under very high disease pressure, the varieties with partial resistance genes such as Janz and Wyalkatchem showed high levels of infection; however yield was significantly greater than in susceptible types. With the partial fungicide protection, significant yield benefits were obtained. In environments which are less conducive to stripe rust, the value of partial resistance would be expected to be greater.

Major gene resistance provides maximum protection from stripe rust, however many of the varieties preferred by Western Australian grain producers utilise some level of partial resistance rather than single gene resistance. In general, partial resistance to stripe rust combined with strategic fungicide application can be used to minimise yield losses and restrict epidemic development.

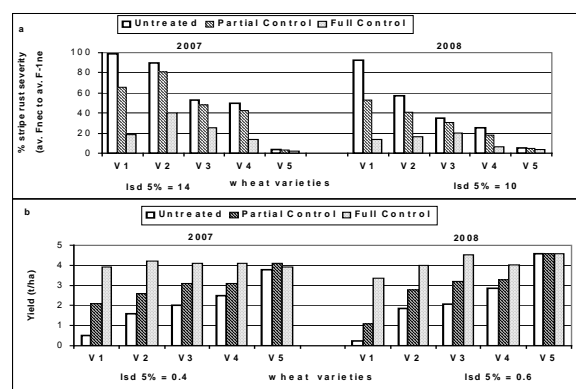


Figure 1. Response of five wheat varieties (V1—EGA Bonnie Rock; V2—Carnamah; V3—Wyalkatchem; V4—Janz; V5—GBA Ruby) with different resistance levels to fungicide application for control of stripe rust. a) average stripe rust severity assessed on two top leaves which showing necrosis due stripe rust infection at milk development stage and, b) yield, in 2007 and 2008 at Manjimup, WA.

ACKNOWLEDGEMENTS

Many thanks to Mr Ian Guthridge, DAFWA, at Manjimup Horticulture Research Institute for help with field operations. This work was supported by Grains Research and Development Corporation (DAW00159).

9 Specific genetic fingerprinting of *Pseudomonas syringae* pv. *syringae* strains from stone fruits in Iran with REP sequence and PCR

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INTRODUCTION

Bacterial canker and blast of stone fruit trees, caused by *Pseudomonas syringae* pv. *syringae* affects all commercially grown *Prunus* species in province of Shiraz in Iran. The relationship between *P. syringae* pv. *syringae* strains infecting *Prunus* species and strains that infect other crops such as, cereals, is unknown and needs to be elucidated. Molecular analysis of genomic variability has been used to differentiate and classify bacterial strains below the level of species repetitive Extragenic palindromic (REP), which are short repetitive DNA sequences with highly conserved central inverted repeats that are dispersed throughout the genomes of diverse bacterial species (1), have been used to design universal PCR primers that generate highly reproducible, strain-specific fingerprints that can differentiate bacterial strains below the level of species or subspecies. The objective of this study was to identify and characterise strains of *P. syringae* pv. *syringae* isolated from various *Prunus* species and other plant hosts by using Rep-PCR analysis.

MATERIALS AND METHODS

Strain isolation. Samples of both healthy and diseased tissues from stone fruit trees were collected from different orchard of Iran

Rep-PCR. Rep primers The PCR conditions were as previously described (21, 32) DNA fragments in the gel were visualised by staining with ethidium bromide.

RESULTS

Twenty-five strains of *P. syringae* pv. *syringae* collected from stone fruit orchard sites, wheat and sugar beet in the Shiraz, Tehran and another part of Iran were used in this study. The DNA fingerprints were determined by using PEP-PCR. Most of *P. syringae* pv. *syringae* strains from stone fruits, shown similar pattern and are different white other hosts. Wheat and sugar beet strain have several common bands.

DISCUSSION

In this study, the *P. syringae* pv. *syringae* strains isolated from *Prunus* hosts in Iran generated similar genetic profiles in PEP-PCR whereas most strains of *P. syringae* pv. *syringae* isolated from other hosts generated dissimilar patterns (3). This suggests a host specialisation of the stone fruit strains within the heterogeneous pathovar *syringae*. Specialisation of *P. syringae* pv. *syringae* strains toward a particular host has been observed in previous studies(4) REP PCR has been shown to be a rapid and reliable method to differentiate plant-pathogenic bacteria at or below the pathovar level with highly reproducible results (5). Our results suggest that strains of *P. syringae* pv. *syringae* that are adapted to a specialised niche, such as Iran stone fruits, may be the result of a recent adaptation and/or genetic isolation, resulting in the genetically homogeneous population of *P. syringae* pv. *syringae* strains from stone fruits observed in this study, which formed a distinct group from strains isolated from other hosts.(3)

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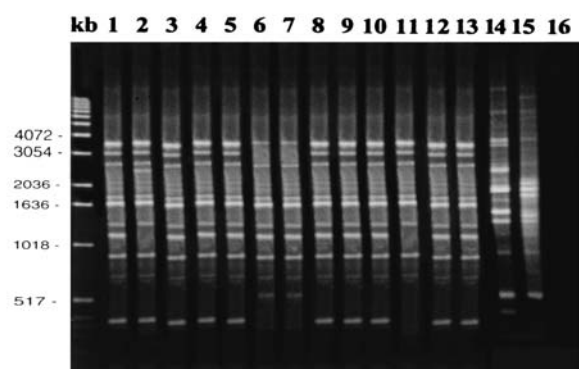


Figure 1. REP fingerprints of several *P. syringae* pv. *syringae* strains isolated from various plant hosts, showing strain variability within the pathovar. Lanes: kb, the 1-kb molecular marker; 1, Almond (pattern 1, 2); 2, Walnut (pattern 3–5); 3, Apricot (pattern 6, 7); 4, Peach (pattern 8, 9); 5, cherry (pattern 10_13); 7, wheat (pattern 14); 8, Sugar beet (pattern 15); 9 Negative control (pattern 16)

85 Non-host resistance and pathogen virulence: an important role of toxic and infection-inducing compound(s) from spore germination fluid of *Botrytis cinerea*

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INTRODUCTION

Plants are continually exposed to a vast number of potential pathogens and, as a result, they have evolved intricate defense mechanisms like hypersensitive response (HR), oxidative burst and increased expression of pathogenesis related protein. The HR appears to play a pivotal role in the success of *B. cinerea*. As a typical necrotroph, it may produce multiple metabolites and proteins that determine its necrotrophic life style (van Kan, 2006). One of the key mechanisms of *Botrytis* species is their ability to induce active cell death in their host plants in order to be pathogenic (van Baarlen et al. 2004). This study describes the action of spore germination fluid of *B. cinerea* (Bc) on pathogenicity and on host responses.

MATERIALS AND METHODS

Spore germination fluid (SGF) was obtained from water-germinated conidia from a highly virulent strain BC.02RO supplemented with 0.05 $\mu\text{g/ml}$ glucose. An avirulent *Alternaria alternata* (15B) and a hypovirulent strain of *B. cinerea* (BC.236795) were evaluated on disease development and cellular response on *Nicotiana benthamiana* (Nb) with or without SGF. Disease development was monitored macroscopically by measuring the lesion area, and, cellular changes were monitored microscopically by histochemical staining. Freeze-dried SGF solution was made as weight/volume. A 40 $\mu\text{g/ml}$ was used as a standard concentration because of visible clear necrosis on Nb, kidney bean, barley, and so on.

RESULTS

A hypovirulent BC.236795 and an avirulent 15B did not infect and evoke visible lesion with sterilised water at 2 dpi on Nb. Both induced papilla formation and callose deposition. On the other hand, BC.236795 or 15B with SGF (40 $\mu\text{g/ml}$) from BC.02RO evoked lesion and cell death on Nb (Fig. 1) as BC.02RO did. The activities of infection-induction and lesion formation are detected in a 10–30 kDa fraction of SGF. That is, BC.02RO-SGF contained toxic and infection-inducing factor(s). H_2O_2 generation was observed 9 h after treatment with SGF (Fig. 1) while the generation was accelerated by inoculation with 15B or BC.236795. These effects were more significant with BC.02RO. The SGF alone induced cell death but not callose deposition. Though necrosis was induced at $>20\mu\text{g/ml}$ of SGF, infection by 15B or BC.236795 was established at $>5\mu\text{g/ml}$ (Fig. 2). Proteinase K (PrK) negated apparently lesion formation and cell death induced by SGF with 15B. Prk also limited lesion formation by BC.02RO dose dependently.

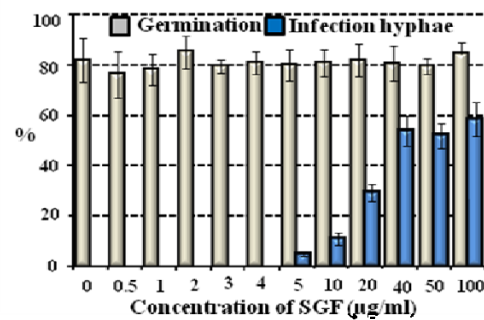


Figure 1. Effect of SGF (40 $\mu\text{g/ml}$) on lesion formation (5 dpi; left), cell death (4 dpi; middle), with or without 15B and H_2O_2 generation (right; 9 h upper, 12 h lower) on Nb.

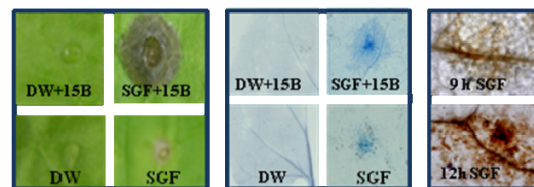


Figure 2. Effect of SGF on infection by 15B on *N. benthamiana* at 2 dpi.

DISCUSSION

As described above, we found that the SGF of a virulent strain of Bc induced necrosis and accessibility even to a hypovirulent Bc or to an avirulent 15B, and both pathogens showed similar activity with exogenous H_2O_2 . The SGF also induced prominent accumulation of H_2O_2 , which is required for cell death (van Kan 2006). It was also reported that accumulated H_2O_2 is necessary to achieve full virulence of *Botrytis* (van Baarlen et al. 2004). Taken together with these reports, we hypothesize that SGF plays a crucial role in establishment of infection or lesion formation by Bc or related necrotrophic fungi mediated by H_2O_2 generation. The preliminary experiment with PrK indicates that the principle for necrosis-induction and accessibility-induction in SGF seems to be a proteinous compound(s). In conclusion, SGF contains determinant(s) of pathogenicity of this necrotroph.

ACKNOWLEDGEMENTS

The research was funded by Japan Society for Promotion of Science.

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51 Impact of *Phytophthora cinnamomi* on native vegetation in South Australia

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INTRODUCTION

Phytophthora dieback, caused by the soil-borne Oomycete *Phytophthora cinnamomi* Rands (Pc), has been identified by the Australian Government as a key threat to native ecosystems. A National Threat Abatement Plan (NTAP) has been developed to limit damage to our native flora and fauna. In spite of the threat that Pc represents for South Australia (SA), basic information about the effect of the disease on native vegetation in SA is lacking. This project aims to increase understanding of the susceptibility of threatened and key plant species and ecological changes in plant communities due to *Phytophthora* dieback in SA.

MATERIALS AND METHODS

Susceptibility testing of threatened and key plant species.

Testing of selected threatened species has commenced (Table 1). These species were chosen on the basis of availability, ease of seed germination and handling in the greenhouse, and occurrence in moderate or high "risk of *Phytophthora*" area(s) (1). Other species, abundant at our field sites, will also be tested, e.g. *Allocasuarina*, *Hakea* and *Hibbertia* spp. Three-month old plants will be inoculated with Pc using a method modified from Butcher *et al* (1984) and Shearer *et al* (2004) and monitored for disease symptoms and mortality.

Table 1. Threatened plant species to be tested for susceptibility to Pc.

Species	Common name
<i>Allocasuarina robusta</i>	Mount Compass oak-bush
<i>Brachyscome diversifolia</i>	Tall daisy
<i>Olearia pannosa</i>	Silver-leaved daisy
<i>Austrodanthonia carphoides</i>	Short wallaby grass
<i>Acacia enterocarpa</i>	Jumping jack wattle
<i>Acacia pinguiifolia</i>	Fat-leaf wattle
<i>Glycine tabacina</i>	Variable glycine
<i>Correa calycina</i>	SA green correa
<i>Pomaderris halmaturina</i>	Kangaroo ls. pomaderris
<i>Prostanthera halmaturina</i>	Monarto mintbush
<i>Oreomyrrhis eripoda</i>	Australian carraway
<i>Spyridium parvifolium</i>	Dusty miller
<i>Spyridium spathulatum</i>	Spoon-leaved spyridium

Dynamics of Pc in the field. The rate and pattern of spread of Pc are being studied at two sites in the Mount Lofty Ranges (Mount Bold reservoir reserve and Scott Creek Conservation Park). The sites are open woodland, are floristically similar to one another and the presence of Pc has been confirmed. Permanent quadrats have been established and the following parameters measured and data collected in 2008:

- soil and fine root samples; baited for Pc
- numbers and health of key indicator species e.g. *Xanthorrhoea semiplana* and other vascular plants
- percentage cover by vascular plants, leaf litter and bare ground
- other data e.g. soil moisture, rainfall.

These parameters will be measured again in autumn and spring of 2009 and 2010.

Effect of companion plants on susceptibility. The hypothesis that the plant neighbourhood influences spread and expression of *Phytophthora* dieback is being examined in a series of pot experiments. In the first experiment, seeds of *Acacia pycnatha* and *A. myrtifolia* have been sown in pots containing 1-year-old plants of *X. semiplana*. Pots will be inoculated with Pc when acacias are 3 months old and symptoms assessed.

Microbial antagonists. Rhizosphere soil from Pc tolerant plants, e.g. some *Acacia* spp., and from sites where Pc is present but not causing disease will be screened *in vitro* for antagonists of Pc, in particular streptomycetes. Preliminary work has yielded several species strongly antagonistic to Pc. Streptomycetes will be tested further for antagonism *in planta*. Results from these experiments may help to explain suppression of Pc root rot in some native ecosystems.

RESULTS AND DISCUSSION

Baseline data collected from the field sites in 2008 will be compared with data collected in 2009 and 2010 which will enable documentation of the rate and pattern of spread of the pathogen and disease over time. Information from field observations and glasshouse experiments about susceptibility of threatened and key species will enable improved management decisions regarding conservation of threatened plant species. Knowledge of companion plant interactions will increase understanding of the factors that affect the spread of the disease. Information from this project will facilitate the adoption of management strategies in line with NTAP objectives.

ACKNOWLEDGEMENTS

This research is funded by the ARC and has the following linkage partners: Adelaide-Mt Lofty NRM Board, Adelaide Hills Council, City of Tea Tree Gully Council, Department for Environment and Heritage, Department of Transport, Energy and Infrastructure, Forestry SA, PIRSA Forestry, SA Murray Darling Basin NRM Board and SA Water. We thank the Sarawak State Government, Malaysia, for funding the PhD studies of Mr Kueh Kiong Hook.

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74 Genetic diversity and population structure of Australian and South African *Pyrenophora teres* isolates

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INTRODUCTION

Net blotch, caused by the fungus *Pyrenophora teres*, is a serious production problem for the barley (*Hordeum vulgare* L.) industry in Australia, South Africa and elsewhere (1, 2, 3, 4). Two forms of net blotch exist: one is the net form (NFNB) caused by *P. teres* f. *teres* (PTT) and the other is the spot form (SFNB) caused by *P. teres* f. *maculata* (PTM). Several Australian and international studies have used molecular markers, such as amplified fragment length polymorphisms (AFLP) to investigate the genetic structure of *P. teres* (3, 5, 6, 7). In contrast, while the incidence of net blotches on barley have increased recently in South Africa, local populations of the fungus have remained uncharacterised. To address this issue, PTT and PTM isolates were collected from the south-western Cape region of South Africa. AFLP analysis was conducted on extracted DNA from these isolates and from a collection of Australian isolates to determine the genetic diversity and structure of South African populations and to determine their relatedness to Australian isolates.

MATERIALS AND METHODS

DNA extractions. Fungal mycelium were harvested from cultures grown on potato dextrose agarose plates at 25°C for one week. A CTAB DNA extraction method was used to extract the fungal DNA.

AFLP analysis. The AFLP procedure was carried out using an Invitrogen AFLP Core Reagent kit. The *EcoRI* primers were hex-labelled. The samples were visualised using a Gel-Scan 2000™ DNA fragment analyser (Corbett Life Sciences, Sydney, Australia).

Scoring and data analysis. Both monomorphic and polymorphic bands were scored and used in the data analysis. Bands were scored independently by two people. The cluster analysis was performed using NTSYSpc V2.20f, whereas the program Structure V2.2 was used to determine the population structure.

RESULTS

AFLP analysis was conducted on DNA of 23 South African and 37 Australian PTT isolates, 37 South African and 29 Australian PTM isolates, six *Bipolaris sorokiniana* isolates, two *P. tritici-repentis* and two *Drechslera rostrata* isolates. Eight primer combinations were used to amplify AFLPs and on average 50 loci were produced with each primer combination. In total, 400 loci could be accurately scored across all samples and 168 of these loci were polymorphic in the *P. teres* samples.

Cluster analysis separated the NFNB and SFNB isolates into two strongly divergent groups (similarity coefficient = 0.6). Low genetic differentiation was observed within the NFNB and SFNB groups (similarity coefficient = 0.9). Interestingly, the South-African NFNB isolates clustered together with the Australia NFNB isolates whereas the South-African SFNB isolates were grouped into a distinct cluster separate from the Australian SFNB isolates.

No genetic differentiation associated with locations within Australia or South Africa could be identified.

The program Structure separated the PTT and PTM isolates into three and two groups, respectively.

DISCUSSION

Our study indicates that the genetic diversity among South African and Australian *Pyrenophora* isolates is low and that there is no clear geographical substructuring. These findings are similar to those of studies in other regions (3, 5, 6). Results produced by the two software packages NTSYS and Structure will be compared and discussed.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Hugh Wallwork and Dr Sanjiv Gupta for the isolate samples provided by them. We also would like to thank Debbie Snyman, Denise Liebenberg and Lizaan Rademeyer for their technical help in the Cengen laboratory. This project was funded by the South African Winter Cereals Trust.

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28 Molecular identification of *Pythium* isolates of ginger from Fiji and Australia

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INTRODUCTION

Pythium myriotylum Drech is one of the main causal organisms for *Pythium* rhizome rot of ginger and is common worldwide. It was first recorded in 1907 by Butler. In a recent review (1) eleven species of *Pythium* were listed as causal agents of a rhizome rot of ginger. *Pythium* affects ginger throughout its growing stages and the main entry points of infection are the buds, roots, developing rhizomes and the collar region of the plant.

In Fiji and Australia *P. myriotylum* is commonly associated with the disease and isolates of this species are capable of destroying ginger rhizomes in 1–2 weeks under appropriate environmental conditions (2). In Fiji, surveys during 2007 and 2008 found that *Pythium* rhizome rot was associated with hot, humid and high rainfall periods during the wet season. It was noted during the previous study (2) that additional species of *Pythium* may be associated with the disease in both countries. Some Fijian isolates grew at different rates on agar and showed variability in cultural characteristics, while preliminary work indicated variability in aggressiveness within isolates obtained from Australia. This abstract details further investigations into the molecular variability of *Pythium* isolates from ginger in Fiji and Australia.

MATERIALS AND METHODS

1. Culture isolation Diseased ginger rhizomes were collected from different ginger growing areas in Fiji (2) and in Queensland. The five Fijian isolates used in this study were imported on an AQIS permit and kept in an quarantine lab at the University of Queensland. Of the Australian *Pythium* isolates used, one was from capsicum and two isolated from diseased ginger rhizomes (Table 1).

2. Molecular identification

2.1 DNA extraction. After 5 days growth on potato dextrose broth, mycelia of each isolate were collected in Falcon tubes and lyophilised. Mycelia were ground in liquid nitrogen to fine powder and DNA extracted as per Lee & Taylor (3).

2.2 Polymerase chain reaction and sequencing. The ITS region of the *Pythium* isolates were amplified using the universal primers ITS 1 (5' – TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC -3') described by White *et al.* (4) and were sequenced at AGRF Brisbane.

RESULTS

Molecular identification BLAST analysis revealed that three *Pythium* species, *P. myriotylum*, *P. vexans* and *P. graminicola*, were identified each from three different ginger growing localities (Veikoba, Navua and upper Naitasiri, respectively). The two Australian ginger isolates revealed sequence alignment with *P. myriotylum* and *P. zingiberis*; the capsicum isolate was confirmed as *P. myriotylum* (Table 1).

Table 1. *Pythium* isolates from Fiji (KRS suffix) and Queensland (BRIP suffix) showing location collected, species identification based on ITS sequence similarity and Genbank accession number where deposited. All isolates from ginger except* from capsicum.

Sample	location	<i>Pythium</i> sp.	GenBank
KRS11	Navua	<i>P.vexans</i>	
KRS13	Muainaweni	<i>P.graminicola</i>	
KRS14	Veikoba	<i>P.myriotylum</i>	FJ797574
KRS15	Waibau	<i>P.graminicola</i>	
KRS17	Veikoba	<i>P.myriotylum</i>	FJ797575
BRIP39907*	Bundaberg	<i>P.myriotylum</i>	FJ797576
BRIP52426	Templeton	<i>P.myriotylum</i>	FJ797577
BRIP52427	Templeton	<i>P.zingiberis.</i>	FJ797578

DISCUSSION

This is the first record of *P. vexans* and *P. graminicola* from ginger in Fiji and the first putative record of *P. zingiberis* in Australia. According to Dohroo (2005) *P.graminicolum* is present in Sri Lanka while *P.vexans* in India. The two species have been found to cause problems during rainy weather. The surveys are still relatively limited and there may be more species present in the Fijian ginger growing areas, or indeed other species present in Fiji and Australia with the capacity to cause rhizome rot in ginger. Consequently, more survey work is warranted. *P. zingiberis* has been recorded in Japan and Korea (5). In Japan, it has been isolated from various parts of rotten ginger, especially from the basal part of terrestrial stem and rhizomes regardless of stages of disease development and locations. It has been isolated from soils where ginger is growing and from areas where ginger has been previously grown. Morphologically, *P.zingiberis* is very similar to *P.myriotylum* and at the molecular level only a single base pair difference in the ITS2 region of the rDNA separates the two species (6). *P.zingiberis* has never been recorded in Australia, and to validate this record further morphological analysis is required.

ACKNOWLEDGEMENTS

This work was supported in part by a John Allwright Fellowship provided to the presenting author. We thank G &AM Stirling and Mike Smith for provision of the Australian isolates and advice in this project.

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29 Development of techniques to measure SAR induction in broccoli for clubroot disease resistance

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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). Salicylic acid 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). An increased concentration of endogenous SA prior to and during SAR has been correlated with an increase in the production of pathogenesis-related proteins throughout the plant. We are investigating SA-induced SAR in broccoli following inoculation with *Plasmiodiophora brassicae*. Molecular and biochemical methods are being developed to measure SAR induction in broccoli for the first time. A real-time reverse transcriptase quantitative PCR (RT-qPCR) has been developed to measure chitinase gene expression in plant tissue. Extraction from broccoli tissue and High Performance Liquid Chromatography (HPLC) analysis are being optimised to quantify SA levels post induction.

MATERIALS AND METHODS

Roots of broccoli (cv. Marathon) seedlings (10 to 14 day old) grown in trays were dipped in SA solution (1–5 mM concentration) for 15 minutes. Root and leaf sample pairs collected from the same plant were harvested 24 hours post treatment. Total RNA was isolated from roots and leaves of each sample for 3 replicates and checked by spectrophotometry for its quality and quantity. RNA was treated with DNase 1 and then reverse transcribed into cDNA and quantified by spectrophotometry. RT-qPCR was conducted in duplicate for each sample using primers for two different genes—Actin-8 (house-keeping gene) and Chitinase (gene of interest). The Delta-Delta C_t method was used for calculating the relative fold change of chitinase gene expression in treated samples compared to the control. SA extraction from broccoli is currently being optimised based on published protocols (3) and quantified using HPLC.

RESULTS AND DISCUSSION

The chitinase gene was chosen for this study as it is a known marker gene for measuring SAR induction and secondly it was possible to design the primers for *B. oleracea*. Following treatment of roots with 1 mM SA chitinase gene expression was increased above controls and was uniform throughout the plant in each of the three replicates. At higher concentrations up to 5 mM chitinase gene expression was less consistent. These results indicate that a low dose of SA might be enough to trigger a good SAR response in the whole plant. A higher dose of SA might not necessarily induce a higher SAR response rather it may alter the physiology of the plant.

Initial HPLC analysis has revealed that SA can be detected at nanomolar levels in broccoli root and shoot extracts post treatment with 1 mM SA (Fig 1). We are now in a position to examine the levels of SA that are present post SAR induction and the response to *P. brassicae*.

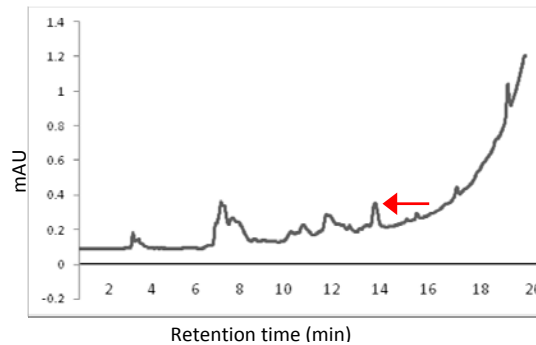


Figure 1. A typical chromatogram of a broccoli root extract that illustrates the retention time of SA (arrow) based on a separation performed on a standard 250mm x 0.5 μ m C₁₈ HPLC column.

These preliminary studies have confirmed that SA can be used as an SAR inducer in broccoli and that the molecular and biochemical techniques under development can be used to measure SAR induction. Future work will determine the link between SA and resistance to *P. brassicae* in broccoli and the optimum level of SA required for SAR induction and disease resistance.

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. We also thank Prof. Jutta Ludwig-Muller, Technical University, Dresden, for her valued expertise at the beginning of this project. D. Lovelock is funded by a DPI-Deakin University Post-Graduate Scholarship.

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52 Reducing the carbon footprint in Riverland vineyards: assessing the efficacy and efficiency of control for powdery mildew by evaluating growers' spray diaries

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INTRODUCTION

A single sulphur spray to control powdery mildew (*Erysiphe necator*) in the Riverland region, comprising ~21,000 ha of viticulture near Loxton, South Australia (SA), costs ~\$1.2 million. Like many other industries, the Australian wine grape industry, competes in international markets. Cost efficiencies and a demonstrably clean and sustainable green image are required. Because recent SA legislation aims to ensure that carbon emission targets are met, the SA wine grape industry is taking the initiative by fostering adoption of the cheapest and environmentally best disease control strategies.

In line with this, we analysed grape grower spray diaries to: 1) assess the efficiency of spraying practices used for powdery mildew control; 2) provide a benchmark for current practice; and 3) identify the scope for introducing modified spraying practices for optimum control using advanced knowledge of disease epidemiology.

MATERIALS AND METHODS

Paper-based spray diaries supplied by two local wineries from 2006/07 were converted to electronic format for the rapid and uniform review of individual records. The records contained vineyard details, spray information (dates, products, application details etc), and winery pre-harvest assessments of powdery mildew severity. A MS Access®-based, vineyard spray program evaluator with a theoretical optimum spray strategy based on best practice spray timing, fungicide treatment, and application technique, was used to: 1) compare each spray record in relation to temporally adjacent sprays; and 2) calculate a disease management score for each record. The scores for each spray event in each diary were then combined for each patch to provide a simple but rapid means of evaluating spray programs used on more than 1,200 patches of Chardonnay, 1,450 of Shiraz and 13 of Verdelho. Of the former, 138 were examined in detail.

RESULTS AND DISCUSSION

Analyses of the spray diaries showed that some growers achieved excellent control with as few as 2–4 sprays while others sprayed ≥ 12 times and achieved poor control. Many applied too many sprays with little benefit. For instance, Chardonnay growers applied an average of 6–7 sprays (range 2–14)/season and 25% applied ≥ 8 sprays annually. Of the total number, 73% (81% of total area surveyed) had at pre-harvest, insignificant amounts of mildew while only 6% (3% area) had levels of disease sufficient for the winery to reject the crop. Significantly, there was no correlation between the number of sprays and the winery's pre-harvest disease score (Figure 1). It was not the number of sprays but their timing that was critical in controlling disease.

A comparison of the disease management scores with the theoretical optimum spray strategy indicated that about 45% of growers were applying well-timed programs whereas 55% were

performing poorly. Nearly all growers were applying the right fungicide treatments but a lack of spray diary data on sprayer calibration, and therefore the efficiency of spray coverage, prevented accurate assessment of spray technique as a factor in disease control. Similarly, the lack of records of inoculum reservoirs inhibited the study of the efficacy of the sprays applied.

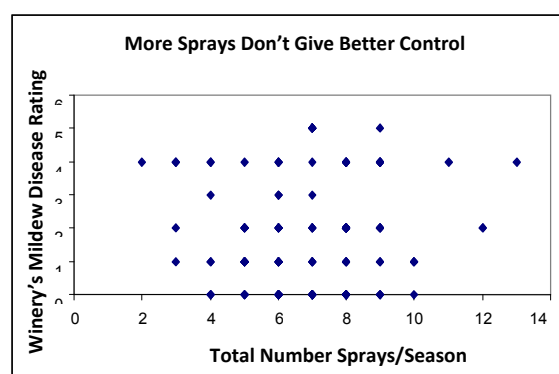


Figure 1. Plot of the number sprays applied for powdery mildew/patch/season for cv. Chardonnay, 2007/08.

Spatial analyses of the spray diary records indicated clustering of vineyards with higher disease scores suggesting that disease control might be improved by addressing cultural, sociological and/or meso-climatic factors. In addition, higher incidences of powdery mildew occurred in patches with under-vine irrigation compared to those with drip irrigation.

Our analyses showed that the vineyard spray program evaluator could compare disease management practices from any spray diary of the same format to: 1) rapidly review past records of sprays applied in other seasons or other regions so that industry bench-marks for the use of fungicides to control powdery mildew can be devised; 2) evaluate planned spray schedules for effectiveness and weakness before sprays are applied in the vineyard; and 3) determine which spray schedules are effective and which are not. From this, the minimum number of effective sprays needed to improve control of powdery mildew can be determined, reducing fuel and chemical use, lowering costs and reducing carbon emissions in Australian vineyards.

CONCLUSION

There is scope to adopt improved spray programs for better control of powdery mildew. The vineyard spray program evaluator could be developed as an online module in *CropWatchOnline.com* for growers, vineyard managers, consultants and others to assess their own records.

ACKNOWLEDGEMENTS

This research was initiated by the Riverland Wine Industry Development Council and supported in part by a Grape and Wine Research and Development Corporation RITA grant.

30 Incorporating host-plant resistance to *Fusarium* crown rot into bread wheat

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INTRODUCTION

Crown rot, caused predominantly by *Fusarium pseudograminearum* (teleomorph *Gibberella coronicola*), is a major soilborne disease problem in the wheat and barley industries. The disease is widespread and causes losses in yield and quality in Queensland, New South Wales, Victoria, and South Australia. Losses are estimated to be up to \$56M in bread wheat throughout Australia. In Queensland, losses have been estimated at up to 50% in some areas and losses of 20 to 30% occur regularly, while the disease can inflict yield loss of up to 89% (1).

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.

Previous 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.

MATERIALS AND METHODS

Of the bread wheat genotypes studied, two (Puseas and Kennedy) are susceptible and seven (2–49, CPI133814, IRN497, Lang, QT10162, Sunco, and W21MMT70) have partial resistance. The parent 2–49 is considered one of the strongest sources of resistance to crown rot currently available (3).

The seedlings were assessed for crown rot resistance in a glasshouse test, following a modification of the Wildermuth and McNamara method (4). This method is a three week duration experiment that closely mimics field infection, and is highly correlated with field results.

The approaches we took were:

- selection in targeted crosses with knowledge of the genetic model
- selection without knowledge of the genetic model
- gene pyramiding using half-sib crosses
- gene pyramiding using molecular tools (DART).

RESULTS AND DISCUSSION

Having the genetic information available enables an informed decision to be made about the difficulty in working with particular crosses.

Many of the crosses in this study had complex epistatic models controlling crown rot resistance. A number were controlled through an additive gene model or additive x additive epistasis, which allows the resistance to be captured in a fixed line. A number of other crosses with strong resistance were controlled with dominance or dominance x dominance epistasis, meaning the resistance will not be able to be captured in a fixed line.

This information is able to guide selection of populations to advance, and explains why resistance in parent lines or segregating material alone will not guarantee resistance will be

found in a fixed line. Further work is under way to compare selection in crosses with different types of epistatic control.

Selection without knowledge of the genetic control can still provide useful results, but until arriving at a fixed line there will be uncertainty about whether the disease resistance is real or the product of unfixable gene interactions.

Half-sib crosses (where the male and female have a parent in common) combining two different sources of resistance, in this case IRN497 and the synthetic wheat CPI133814, were crossed into a common agronomic background (Sunco). This can be a useful method of elevating resistance by combining diverse resistance genes. Resistance levels in the progeny are extremely high after three rounds of selection (F2 to F4), with the best showing ~30% less disease severity than 2–49.

DART markers have been used to direct intercrosses between resistant selections from a cross of CPI133814 and IRN497, which was identified as the optimal cross for strongest crown rot resistance (from the listed parent set). DART genotyping can identify gene differences in individuals that show the same level of resistance, enabling crosses to be made to maximise the amount of resistance genes within an individual plant. This strategy is aiming to produce a parental line for further development with elevated resistance levels beyond those currently available, rather than a variety for release, as the parents lack adaptation characteristics.

Pre-breeding selection work has commenced with the better performing crosses that include an adapted parent in the cross.

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31 Management and distribution of huanglongbing in Pakistan

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INTRODUCTION

Citrus as a crop provides living for individual farmers and foreign exchange for Pakistan as it is one the major agricultural export commodities. This fruit crop is susceptible to many biotic stresses including diseases of which huanglongbing is perhaps the most devastating of all. The presence of this disease has previously been reported in Pakistan, however, the molecular evidence that it is caused by '*Candidatus Liberibacter asiaticus*' (α -Proteobacteria) was provided only recently (1). Both the prevalence and severity of this disease are, however, yet to be ascertained. This information is imperative to devise a management strategy at national level for containing the losses sustained by the farmers. Farmers, as well as extension workers, also need to be made aware of the presence of, and damage caused by, this disease and a concerted extension campaign is required for this purpose. Due to the technical difficulties in the diagnosis of the disease, a diagnostic service should be provided to the farmers at more than one place in the country. To enable the medium equipped laboratories a testing kit can be useful. These points have been addressed in the present study.

METHODOLOGY

The Survey and Sampling Strategy. To assess the prevalence and severity of the disease, a survey and sampling strategy has been devised which we term as "triple five", to survey the citrus orchards country wide and collect the suspect samples based upon visual symptoms.

Pakistan is administratively divided in to four provinces (Punjab, Sindh, Balochistan and Sarhad or North West Frontier Province) and four territories (Islamabad Capital Territory, Federally Administered Areas (FATA), the Northern Areas (FANA), and Azad Kashmir). The provinces of Sarhad and Balochistan each have Provincially Administered Areas (PATA) as well. Each province or territory is further subdivided in to districts and tehsils. The citrus orchards are spread around the country with a few areas with dense plantings and others with little production. Based upon existing statistics, districts of Pakistan containing citrus orchards covering at least 100 hectares were selected for the survey. At least five orchards in each tehsil were surveyed to select five trees on the bases of visual symptoms. Five symptomatic leaves were collected from each tree and tested for the presence of the disease. Projects are also under way in these areas to eventually uplift the farmers' income from citrus. These samples have been tested by both PCR and the iodine-starch test (IS (2 and 3)).

RESULTS AND DISCUSSION

The Incidence of HLB. Survey of over 12 districts in Punjab has been completed and over 400 samples tested for the presence of HLB. Usually, the IS and the PCR test results were in agreement. However, some samples showing positive IS tests were found to be negative by PCR. The data will be provided later during the presentation.

Multiplexing of primers for '*Ca. Liberibacter asiaticus*' with those for '*Ca. L. americanus*' has been successful; however, '*Ca. L. americanus*' has not been detected.

Huanglongbing Map of Pakistan. Currently, a citrus map of Pakistan is being prepared based upon the available statistics about the fruit crop. This citrus map of Pakistan will be upgraded to the huanglongbing map of Pakistan based upon the results of this study. Year-round meteorological data will be incorporated showing highest temperature in the areas in Pakistan.

Extension activity. Two brochures have been published for the awareness of the extension workers and the local farmers each in English and Urdu disseminated. The same brochure is now planned to be expanded to include all the prevalent diseases and insect pests of citrus as well as agronomic and postharvest recommendations which will eventually lift economic circumstances of the individual farmers.

Building nation-wide diagnostic capability and capacity. The presence of HLB is detected by the following methods: the presumptive IS test and by PCR. A kit has been prepared containing all the ingredients required to do these tests. The Part A of the kit has the ingredients for the IS test. Part B of the kit has the ingredients used for DNA release and PCR analysis including a 1 tube PCR method. This kit is currently in the final stages of its testing and will be released to the interested parties soon. Any laboratory with a standard thermal cycler and gel electrophoresis facility would be able to test the presence of disease using this technique. Therefore, it will be a great boost for diagnostic capacity building in the country. Training workshops are also planned for this purpose.

Huanglongbing Testing Services. One of the main sources of the disease is budwood from the infected mother blocks. Most of the budwood is provided from the government-owned or administrated citrus orchards. Screening these blocks for the presence of this disease will greatly help in suppressing the spread of HLB through infected budwood material. Therefore, we have established a service for the testing facility of these mother blocks.

The service is also offered to general farmers and documentation has been prepared to educate them with the procedures of taking and posting samples for testing.

These services will remain free of charge as long as funds remain available for this purpose.

Seasonal Variation. Extremely high temperatures may restrict proliferation of the pathogen in infected trees. Data is currently being obtained from the Meteorological Department of Pakistan to find out the highest temperature range in the country. This information will be incorporated in to the citrus map of Pakistan. This map will indicate relationship between the disease and high temperatures in the citrus growing areas of Pakistan.

Effect of Temperature on the Pathogen and its Remaining DNA in Infected Leaves. The leaves from the same infected twig are being subjected to heat treatments of different temperatures and times, then tested for the presences of pathogen DNA using standard and real-time PCR protocols to ascertain the concentration of DNA within the leaves.

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75 The *Fusarium oxysporum* f. sp. *ubense* tropical race 4 vectoring ability of the banana weevil borer (*Cosmopolites sordidus*)

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INTRODUCTION

Fusarium wilt of banana is regarded as one of the most widespread and destructive plant diseases in the recorded history of agriculture (1). *Fusarium* wilt is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *ubense*, *Foc*. *Foc* is present throughout the world where bananas are grown. A particularly virulent strain capable of attacking Cavendish bananas in the tropics and referred to as tropical race 4 (*Foc* TR4), was discovered in 1997 in the Northern Territory. It has since led to the closure of several banana plantations (2). To date, *Foc* TR4 does not occur in any other state of Australia. *Foc* TR4 is capable of killing plants faster than any other strain and disease can build up rapidly without control measures (3). There is no method for eradicating the fungus. Current control measures involve limiting the spread of the pathogen within and between farms. However, these measures have often been ineffective and disease has continued to appear in new areas. The reasons behind this spread have not always been easy to explain.

The banana weevil borer, *Cosmopolites sordidus*, is present in most banana production areas throughout the world. It causes damage to banana plants by boring into the rhizome and pseudostem to feed and lay eggs (4). Weevils are capable of crawling between banana plantations. Therefore, it is important to know if they are capable of spreading *Foc* TR4.

This project aims to determine the presence of *Foc* TR4 on or in banana weevils. This is highly important, especially if this pathogen is detected in areas of Australia or other countries where it currently is not present. Revealing whether banana weevils are potential vectors of *Foc* TR4 will provide a greater understanding of disease epidemiology and could assist in limiting spread.

MATERIALS AND METHODS

Pseudostem traps were set at a *Foc* TR4 infested research site, the Coastal Plains Banana Quarantine Station (CPBQS), as well as a site free from *Foc* TR4. A total of 50 weevils were collected from the traps at CPBQS, as well as ten control weevils from the site not infested with *Foc* TR4. They were individually vortexed for in sterile distilled water (SDW) to loosen the fungal spores on the external section of the weevil. After vortexing the insects were surface sterilised before the internal sections were macerated in SDW. The SDW solutions from both the 'external' and macerated 'internal' sections were spread onto plates of malachite green agar. Fungal growth was subcultured onto potato dextrose agar and single spore isolates were obtained. DNA was extracted from putative *Foc* TR4 fungal growth and analysed using *Foc* TR4 specific primers to confirm the fungus identity.

RESULTS

All the macerated internal sections of the weevils collected from the CPBQS tested negative for *Foc* TR4 fungal growth. However, viable spores were detected from the suspension created by the external sections of five of the 50 weevils by PCR analysis. None

of the ten control weevils contained *Foc* TR4 spores, either internally or externally (Table 1).

Table 1. Presence of *Foc* TR4 from *Cosmopolites sordidus*

Trapping site	No. of weevils analysed	Body part(s) analysed	Recovery of <i>Foc</i> TR4 (%)
<i>Foc</i> TR4 infested	50	Internal	0
		External	10
Not infested (control)	10	Internal	0
		External	0

DISCUSSION

While *Foc* TR4 was not successfully detected in the internal sections of the weevils, it was detected on the external sections. These preliminary results imply that *C. sordidus* can act as a carrier for *Foc* TR4 and possibly assist with its dispersal within and between plantations. *C. sordidus* may also vector other strains of *Foc* present in Australia and throughout the world.

ACKNOWLEDGEMENTS

The authors would like to acknowledge The Department of Agriculture, Fisheries and Forestry for providing the funds for this project.

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76 Genetic diversity of *Pseudocercospora macadamiae* populations by PCR-RFLP

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INTRODUCTION

Pseudocercospora macadamiae is known only to exist and cause husk spot of macadamia in commercial orchards in Australia (1, 2). Field trials and observations suggest that the asexual conidia of the fungus are the most important infective propagule in the disease cycle (3). The teleomorphic state of *P. macadamiae* is yet to be observed in nature or culture (4). We hypothesise that the husk spot disease cycle lacks a teleomorphic state of *P. macadamiae*.

Testing the hypothesis that a teleomorph is absent from the husk spot disease cycle by means of direct survey of orchards or native vegetation is challenging. However, if the teleomorph is absent from commercial orchards, it is expected that the lack of sexual reproduction would result in predominantly clonal populations of the fungus. Therefore, we investigated the genetic diversity of field populations of *P. macadamiae* to test our hypothesis.

MATERIALS AND METHODS

In order to determine the genetic diversity of *P. macadamiae* populations, 105 isolates were collected from diseased fruit from trees in three orchards located at Bundaberg (Qld), Glasshouse Mountains (Qld), and the Northern Rivers (NSW), respectively. DNA was extracted and PCR-RFLP performed in duplicate for 6 genes (actin, β -tubulin, calmodulin, EFA, G3P and ITS), each digested with three restriction enzymes.

RESULTS

Results of the PCR-RFLP study showed that more than 80% of the isolates were of the same genotype, with the predominant genotype occurring at frequencies of 64, 95, and 79% at Bundaberg, Glasshouse Mountains and Northern Rivers, respectively (Fig 1). The Northern Rivers population included the highest number of genotypes for a single location (5). The number of polymorphic alleles differentiating the identified genotypes was low. Of the six genes studied, actin, β -tubulin, and EFA were the most polymorphic, whilst no polymorphisms were detected in the calmodulin, G3P or ITS genes.

DISCUSSION

We hypothesised that the husk spot disease cycle lacks a teleomorphic state of *P. macadamiae*. Our study shows the genetic diversity of *P. macadamiae* populations to be low, and that a single genotype predominates at all the collection sites. The lack of genetic diversity is supportive of our hypothesis, and evidence for any significant role of a teleomorph in the husk spot disease cycle remains elusive.

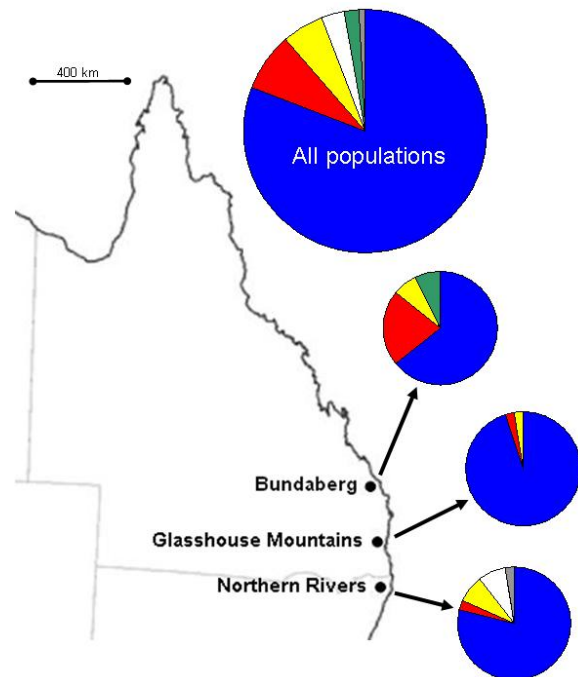


Figure 1. Location of *Pseudocercospora macadamiae* collection sites and pie charts representing the genotype frequency within individual, and all, populations. Different segments of pies represent unique genotypes.

ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support of Horticulture Australia Ltd., Australian Macadamia Society Ltd., and The University of Queensland.

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32 Investigating the potential of in-field starch accumulation tests for targeted citrus pathogen surveillance in Australia

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INTRODUCTION

Australia needs to be prepared to undertake large-scale surveillance for the devastating, exotic disease of citrus, huanglongbing (HLB) (*Candidatus Liberibacter* species). One immediate obstacle is the potential for endemic diseases to exhibit HLB-like symptoms, such as Australian citrus dieback (ACD) (possibly phytoplasma), and tristeza (*Citrus tristeza virus*) (CTV). Similar leaf symptoms include asymmetric mottling/chlorosis, vein yellowing and corking. In addition, effects of nutrient deficiency, wounding and other maladies can also be similar, adding to potential confusion. Because of this, the number of specimens collected based on visual symptoms would likely overwhelm current diagnostic capacity, and a rapid, in-field test to help select diagnostic samples is needed.

Starch accumulation has been well correlated to HLB symptoms in some studies (1–3) and could be a useful tool to aid surveillance. However, starch can accumulate due to nutrient deficiencies, insect damage and other factors including diseases other than HLB. Therefore, a preliminary study was undertaken to: i) compare two established starch tests; and ii) postulate the specificity of the starch tests amongst endemic citrus diseases.

MATERIALS AND METHODS

Citrus trees (grapefruit, mandarin, lemon, pommelo) and one tree of *Murraya* sp. were surveyed in the Burnett Basin, Queensland. Leaves showing asymmetric mottling and/or chlorosis, vein yellowing and/or corking, and corresponding healthy leaves were sampled. Leaves were field tested for starch accumulation through its reaction with iodine. The iodine reactions were carried out using both the “scratch” (2) and “leaf cut” (3) methods. The leaf cut method immersed the freshly cut edge of a leaf into iodine solution, then required inspection of the cut edge with a hand lens to visualise any inky blue/black colour development. The scratch method involved abrading the leaf surface with sandpaper, and then transferred the sandpaper to a ziplock bag containing an iodine solution. Any colour change was observed in the bag.

Leaf samples were tested in the laboratory for pathogens capable of causing the symptoms of interest. Direct tissue blot immunoassay was used to detect CTV and molecular methods were used to detect phytoplasmas (ACD) and ‘*Ca. Liberibacter*’ species causing HLB.

RESULTS

In total 31 samples (20 symptomatic and 11 healthy) were collected and tested. No healthy samples were positive for starch accumulation by either method. Positive, partial, and negative results were found amongst the symptomatic samples (Table 1). Variation between methods was least amongst positive samples, according to either method, with 3 out of 5

possible samples in agreement (60%). The greatest variation between the two methods was in the production of ambiguous results, with only 1 of 13 possible samples being in agreement (8%).

Table 1. Starch accumulation Number of symptomatic samples positive, partial or negative for starch accumulation, determined by scratch or leaf cut methods, and the number of samples for which the methods agree.

Starch reaction	Scratch	Leaf cut	Agreement
Positive	5	4	3
Partial	2	13	1
Negative	13	3	3

The six positive starch reactions (by either method) could not be clearly attributed to any particular pathogen. However, one leaf sample was taken from a wounded branch, and another noted as possible early symptoms of *Mycosphaerella citri*. The causal agent of HLB, *Ca. Liberibacter* was not detected in any samples using the laboratory diagnostic assays. CTV was detected in 23 leaf samples, 7 of which were asymptomatic, healthy leaves. An additional 3 leaves had symptoms typical of ACD, tested positive for phytoplasmas, but had negative/partial starch reactions.

DISCUSSION

Starch accumulation was detected in Australian citrus leaf samples using both methods and could not be attributed to any of the pathogens tested for. This suggests that the test may be of limited use for pre-HLB incursion surveillance. If starch accumulation can be demonstrated to be useful post-incursion (when HLB might be responsible for the majority of starch accumulation), the ‘scratch’ method is preferred due to its delivery of clear results and practicality for field use. Individual sampling kits for the scratch method can be easily pre-prepared, the results photographed for record keeping, and the waste self-contained for removal from the survey site and disposal.

ACKNOWLEDGEMENTS

Assistance of Cherie Gambley is gratefully acknowledged.

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77 Priming for resistance against pathogens: cellular responses of Arabidopsis to UV-C radiation

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INTRODUCTION

Plants face many biotic and abiotic challenges in the environment including pathogen challenge and damage due to energetic wavelengths of light in the ultraviolet (UV) region, both of which have detrimental effects on plants.

When *Arabidopsis thaliana* was irradiated with doses of UV-C light between 0 and 500 J/m², leaves showed resistance towards compatible isolates of the Oomycete *Hyaloperonospora arabidopsis* in a dose-dependant manner (1). Previous research has strongly suggested that this priming response is linked to DNA damage and repair, both of which are invoked after UV-C irradiation (2).

It is still unclear, however, which signals and biochemical events regulate this induced defence response and how they may relate to DNA damage/repair. Also, it is still not known if the same phenomenon can be used to induce resistance against other pathogens or whether UV radiation may be having more subtle non-targeted effects on host cells. This study examines the cellular and tissue responses of Arabidopsis leaves following exposure to doses of ultraviolet radiation.

MATERIALS AND METHODS

UV-C treatments. Arabidopsis Col-0 plants were irradiated with different doses of UV-C (500 and 1000 J/m²). Plants were then returned to normal growth conditions. Leaf tissue was harvested at 0 and 24 hours post irradiation.

Callose and reactive oxygen species assays. Leaves were stained for callose deposition with aniline blue (0.5%) and visualised using fluorescence microscopy. The production of hydrogen peroxide was visualised using 3, 3 diaminobenzidine (DAB).

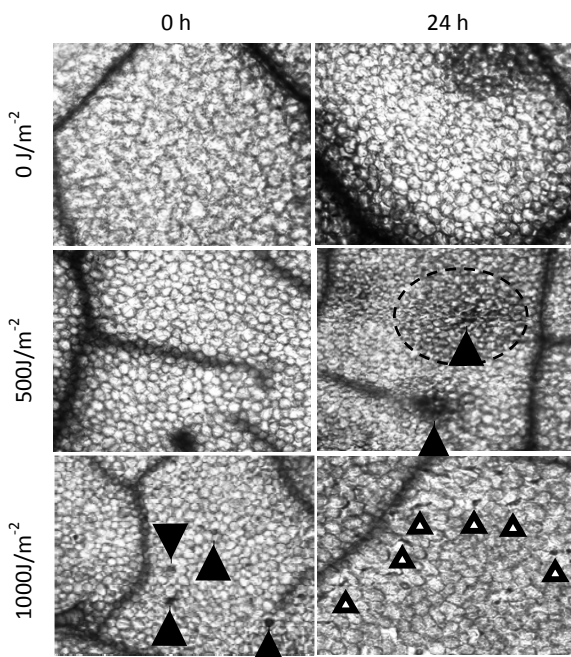


Figure 1. UV-C treated leaf tissue stained with DAB, dark spots indicate the presence of H₂O₂ within cells (solid arrows). Dead cells can be seen as stained collapsed cells (unfilled arrows).

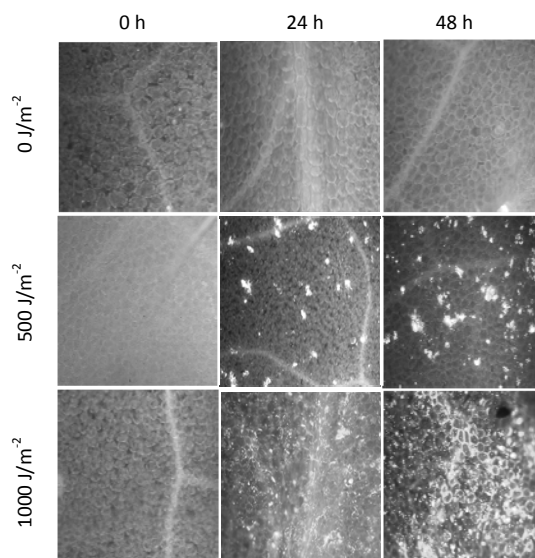


Figure 2. Leaf tissue stained with aniline blue, no callose is present in controls but at 500 and 1000 J/m² callose was induced at 24 and 48h (bright areas in the figure).

RESULTS AND DISCUSSION

H₂O₂ production increased following UV treatment (Figure 1). Lack of DAB staining in the control showed low levels of H₂O₂ to be present. However, at 500 J/m² increased H₂O₂ production at 24 hours was observed. Increased H₂O₂ accumulation could also be seen in leaves exposed to 1000 J/m² at 0 and 24 hours. H₂O₂ production was associated with collapsed cells that appear to have undergone cell death.

UV-C radiation stimulated the deposition of callose in cells and cell walls in both a time and dose dependent manner (Figure 2). Deposition of callose was most intense at 1000 J/m² but less so for treatment at 500 J/m² at both 24 and 48 hours. Future work aims to determine if the induced resistance seen in interactions of Arabidopsis with *H. arabidopsis* occurs in other interactions of pathogens with different lifestyles (eg. biotrophs, hemi-biotrophs and necrotrophs).

ACKNOWLEDGEMENTS

We thank the Australian Research Council for funding.

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11 Boneseed rust: a highly promising candidate for biological control

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INTRODUCTION

The woody evergreen shrub boneseed (*Chrysanthemoides monilifera* ssp. *monilifera*), which originates from South Africa, is a major invasive plant of natural ecosystems in Victoria and South Australia. Small infestations also occur in Tasmania. Improving the effectiveness of the biological control program against boneseed has been identified as a high research priority in the National Strategy for this Weed of National Significance, as none of the six insect agents released so far have established in the field.

The systemic South African rust fungus, *Endophyllum osteospermi*, is a highly promising biological control agent for boneseed because it reduces growth and reproduction of plants by causing extensive deformation of infected branches (witches' brooms). In South Africa the rust appears to be a primary cause for the decline and death observed in some local boneseed populations (1, 2). The systemic nature of the rust is a desirable characteristic for biological control purposes as once the fungus is established within the host the infection is retained until the death of the witches' brooms. The boneseed rust is only recorded in South Africa on a small group of related plants of the genera *Chrysanthemoides* and *Osteospermum* (Calenduleae: Asteraceae). As there are no indigenous representatives of the Calenduleae in Australia, the non-target plants most at risk from this rust fungus are the introduced, ornamental species belonging to this tribe.

A novel approach had to be taken to test the host-specificity of *E. osteospermi*, because it develops visible symptoms only 1–3 years after infection of its host. We present in this paper results from host-specificity tests so far.

MATERIALS AND METHODS

Host-specificity tests. Tier 1 tests were performed on detached leaves of plant species of the approved test list to determine, using microscopy techniques, whether the rust was capable of penetrating epidermal cells of non-target species. Additional Tier 1 tests were also carried out on leaves still attached to plants of some of the species.

Tier 2 tests on leaves still attached to plants of the species where penetration occurred in Tier 1 tests were performed to determine if the fungus was capable of colonising tissue of these species in the weeks following inoculation.

RESULTS

In Tier 1 tests, successful penetration was observed on boneseed and its close relative species tested within the Calenduleae tribe (bitou bush [*C. monilifera* subsp. *rotundata*], *Osteospermum* spp., *Dimorphotheca jucundum*) (3). Penetration also occurred on four other species outside the Calenduleae (*Gazania rigens*, *Gerbera jamesonii*, *Bedfordia arborescens*, *Eucalyptus cladocalyx*). Additional Tier 1 tests carried out on leaves still attached to plants confirmed accuracy of results obtained with detached leaves. Penetration of epidermal cells however, does not necessarily imply that the infection process will continue and be successful.

Three series of Tier 2 tests were performed in South Africa and in the CSIRO quarantine facility in Canberra. No leaf colonisation was observed on boneseed and any of the other species tested. These results cast doubts on the belief that the fungus colonises plants via young leaves. It is possible that infection of axillary buds is essential for further colonisation. Alternatively, conditions during tests may have been slightly suboptimal and prevented infection to occur.

DISCUSSION

The difficulties encountered with Tier 2 tests prompted us to initiate a series of Tier 3 tests, whereby whole plants are repeatedly inoculated with the rust fungus over a few weeks and maintained for up to 2–3 years until witches' broom symptoms developed. These tests were conducted in late winter 2008 and witches' broom symptoms have not yet developed on boneseed or any other species inoculated.

Results from host-specificity tests will be used to fully assess the risk of significant impact on non-target plant species, before deciding if an application for the release of this rust fungus in Australia should be submitted to the authorities.

ACKNOWLEDGEMENTS

We thank Ms Gwen Samuels (PPRI) and Melissa Piper (CSIRO) for technical assistance. Financial support from CSIRO, Australian and New Zealand Environment and Conservation Council (ANZECC) and Land and Water Australia (Defeating the Weed Menace R&D initiative) is gratefully acknowledged.

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10 Can additional isolates of the Noogoora burr rust fungus be sourced to enhance biocontrol in northern Australia?

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INTRODUCTION

Noogoora burr (*Xanthium occidentale*) is an invasive plant across northern Australia, mostly inhabiting riparian areas. The exotic rust fungus *Puccinia xanthii*, illegally or accidentally introduced to Australia in the mid-1970s, has been highly effective in controlling Noogoora burr in south-eastern Queensland, but has had limited impact in tropical northern Australia (1). The introduction of additional isolates of this rust fungus better adapted to tropical conditions has been suggested as an approach to improve impact of this biological control agent in far northern regions (2).

We present results from 1) surveys carried out in tropical America, 2) pathogenicity tests with Australian and exotic isolates of *P. xanthii* performed on Australian accessions of Noogoora burr and other *Xanthium* spp. and 3) the identification of a diagnostic marker that differentiates between Australian and exotic rust isolates.

MATERIALS AND METHODS

Surveys. Surveys for exotic isolates of *P. xanthii* were undertaken in 2007 in Venezuela, Mexico and Dominican Republic, areas that climatically match those of northern Australia where the rust fungus has not been highly effective (2). Rust-infected material collected was pressed and dried and then placed at 4°C until shipment to the CSIRO quarantine facility in Canberra.

Pathogenicity tests. Noogoora burr plants (ex. Daly River, NT) were inoculated with telia (3) collected from each of the overseas sites and from two purified Australian rust isolates (ex. Daly River and Victoria River, NT). This trial was repeated once. Additional inoculations of other Australian accessions of Noogoora burr and other *Xanthium* spp. were also performed.

Diagnostic marker. Primers were designed from characterised Simple Sequence Repeats (SSR) loci isolated from *P. xanthii*. Primers were screened on the DNA of 29 single-telium isolates from Australia, Hungary, Brazil, Argentina, Mexico and Dominican Republic.

RESULTS

Surveys. *P. xanthii* was found at 9 of the 13 *Xanthium*-infested sites surveyed in Dominican Republic, and at three of the four infested sites in Mexico. None of the plants of the two *Xanthium* populations found in Venezuela were infected by the fungus.

Pathogenicity tests. None of the Australian accessions of Noogoora burr and other *Xanthium* spp. inoculated with the exotic rust isolates developed disease symptoms, even though germination tests indicated the inoculum was viable. Microscopic examination of cleared and stained leaf samples from these plants showed typical plant resistance responses following penetration by the rust. In all trials, plants inoculated with the two Australian rust isolates developed disease symptoms. Closer examination of *Xanthium* specimens collected in Dominican Republic and Mexico revealed that they were

morphologically and genetically different to Australian accessions (data not shown).

Diagnostic marker. Several of the eight SSR markers identified differentiated rust isolates from Australia, Mexico and Dominican Republic, with SSR *px09* being the most reliable diagnostic marker.

DISCUSSION

This body of work indicated that Australian Noogoora burr plants, as well as other *Xanthium* spp., are resistant to *P. xanthii* isolates collected in Dominican Republic and Mexico. This came as a major surprise considering previous research showed that an Australian isolate of the rust was capable of infecting the four different *Xanthium* species found in the 'Noogoora burr complex' in Australia: *X. occidentale*, *X. italicum*, *X. orientale* and *X. cavanillesii* (3). As a result, it was not possible to establish cultures of the exotic rust isolates for subsequent host-specificity tests.

SSR marker *px09*, could be developed into a simple PCR-based diagnostic tool to differentiate between exotic and Australian isolates of *P. xanthii*. Such a tool would be valuable for monitoring the establishment of additional isolates of *P. xanthii* from tropical America after their release in Australia, providing that isolates pathogenic to Australian Noogoora burr plants can be found in the future.

A more extensive follow-up project is required to deliver on the original goal of introducing additional isolates of *P. xanthii* better adapted to the climate of tropical northern Australia. The establishment of an outdoor experimental garden consisting of northern Australian accessions of Noogoora burr in tropical America would be required to source pathogenic rust isolates.

ACKNOWLEDGEMENTS

We are grateful to collaborators in Queensland, Northern Territory and NSW for collecting and sending seeds from various Noogoora burr infestations. Financial support from CSIRO, DAFWA and Land and Water Australia (Defeating the Weed Menace R&D initiative) is gratefully acknowledged.

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12 *Helicotylenchus* nematode contributing to turf decline in Australia

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INTRODUCTION

Helicotylenchus species (spiral nematodes) are the most commonly detected plant-parasitic nematodes found in turf samples. These nematodes are generally considered as migratory ectoparasitic and/or migratory semi-endoparasitic feeders, that is, they feed from outside the roots or partially embedded inside the roots. Damage caused by these nematodes is light to dark brown or reddish brown necrotic lesions. The aim of this work was to identify the spiral nematode species causing turf decline in Australia.

Turf samples were submitted to the Crop Health Services, DPI Victoria by green keepers and turf consultant companies for the diagnosis and assessment of plant-parasitic nematodes associated with turf decline. These samples were from all mainland states as well as Tasmania and collected during 1996–2008.

Helicotylenchus species can be difficult to identify and require comparison of characters such as tail shape, body on death, position of vulva and shape of lip region. Morphometrics are also used which include body length, stylet length, tail length and position of phasmids in relation to the anus.

METHOD

Extraction and Fixing. Nematodes were extracted from 200ml soil samples using the Whitehead tray technique, incubated at room temperature about 25°C for 48 hours. The specimens were collected in a 38µm sieve, fixed in 4:1 formalin-acetic acid fixative and then processed through an alcohol/glycerol series to be mounted in glycerol on permanent slides. The slides were deposited in the Victorian Plant Pathology Herbarium, DPI Victoria.

RESULTS

Identification. The main morphological characters on which the identifications are based are presented in Table 1. 3,812 samples were received from bowling and golf greens for counts and identification of plant nematodes. Figure 1 shows New South Wales had the most samples from identified locations compared to other states (Fig.1).

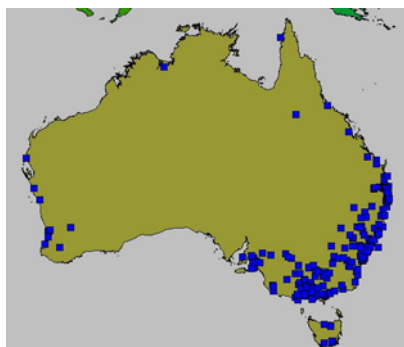


Figure 1. Distribution of *Helicotylenchus* in Australia

The highest numbers per 200ml soil were detected from South Australia and lowest from Tasmania. The numbers of *Helicotylenchus* species present in each sample varied from an

average of 393–6,345 (Table 2). The specimens found in turf samples were identified as *Helicotylenchus pseudorobustus*, *H. dihystrera* and *H. erythrinae*. The most commonly found species was *H. pseudorobustus* and also it was recorded for the first time on turf in Australia. There were occasions when more than one species of *Helicotylenchus* was identified in a sample.

Table 1. Morphological characters used to identify three species of *Helicotylenchus* from turf samples.

<i>Helicotylenchus</i> species	Body length (µm)	Tail shape	Lip Annule	Position of phasmid
<i>H. pseudorobustus</i>	706–822	Tail with distinct smooth ventral projection	4–5	5–8 annules anterior to anus
<i>H. dihystrera</i>	610–860	Tail with indistinct smooth ventral projection	4–5	6–12 annules anterior to anus
<i>H. erythrinae</i>	480–610	Tail with distinct pointed ventral projection	6–12	2 annules posterior, 4 annules anterior to anus

Table 2. Number of identified locations, samples and average of *Helicotylenchus* spp detected from various states of Australia.

States	No. bowling and golf greens	Number of samples	Average number of <i>Helicotylenchus</i> per sample (200 ml soil)
SA	23	139	6,345
VIC	90	289	1,234
NSW	146	854	870
QLD	34	116	1,662
WA	10	25	613
TAS	7	11	393

DISCUSSION

The threshold damage caused by spiral nematodes on turf has been calculated as 600 nematodes per 200ml soil. Of the total 1955 *Helicotylenchus* detected samples, 30% of these were above the damage threshold. *Helicotylenchus* spp were found to be associated with declining couch grass (*Cynodon dactylon*) and for the first time in bent grass (*Agrostis tenuis*). Further survey work is required to investigate the distribution of each identified species and their role in turf decline in Australia.

78 The effect of phosphonate on the accumulation of camalexin following challenge of *Arabidopsis* by *Phytophthora palmivora*

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INTRODUCTION

Potassium phosphonate (phosphonate) is commonly used to prevent and treat disease caused by various species of *Phytophthora*. Phosphonate acts by enhancing the innate defence response of the host plant (1), although the precise mechanisms remain unknown. *Arabidopsis thaliana* provides an opportune model for the study of plant-pathogen interactions and when inoculated with *P. palmivora*, as phosphonate application induces an incompatible defence response (2). Part of this interaction may include the accumulation of the phytoalexin camalexin, however *A. thaliana* mutant studies suggest that camalexin is not always required for resistance. By assessing the effect of phosphonate at individual steps of the camalexin biosynthesis pathway, we aim to learn more about the phosphonate-induced defence response and its role in pathogen restriction.

METHODS

Plant Material and Inoculations. Approximately 0.2mg of *A. thaliana* seed was added to each 125mL conical flask containing half strength MS supplemented with 20% sucrose with or without 100 mg/L phosphonate, pH6.5 (Agri-Fos 600; Agrichem). Each flask was stoppered then shaken (120 rpm) under 12h light/dark at 24°C. After 21 days seedlings were inoculated with zoospores of *P. palmivora* (2).

Extraction and analysis. Camalexin was sampled at 0, 12, 24 and 48h post-inoculation (pi). Approximately 0.3g of macerated tissue was boiled in 80% methanol (20 min, 65°C) then stored overnight at 4°C. Samples were centrifuged and the liquid fraction removed and evaporated. The residue was extracted three times with chloroform and the pooled chloroform fraction was evaporated to dryness. The pellet was dissolved in 500uL of 50% menthol then samples were filtered. Camalexin was quantified utilising a C18 column on a Dionex HPLC system and verified with synthetic camalexin (kindly provide by Jane Glazebrook, University of Minnesota). Data were transformed if required and analysed using ANOVA.

RESULTS AND DISCUSSION

Camalexin accumulation in all treatments rapidly increased between 12 and 24h pi, and then decreased between 24 and 48h (Figure 1). There were no significant differences between any of the treatments ($p=0.058$), however the greatest increase in camalexin accumulation followed inoculation, whether plants had been treated with phosphonate or not.

The 'peak' in camalexin accumulation at 24h has been reported in several ecotypes of *Arabidopsis* and can result from either biotic or abiotic factors (3). Of all treatments, we found that inoculated seedlings had the highest level of camalexin accumulation between 24–48 h pi. This supports the identification of camalexin as a phytoalexin, produced in response to pathogen attack.

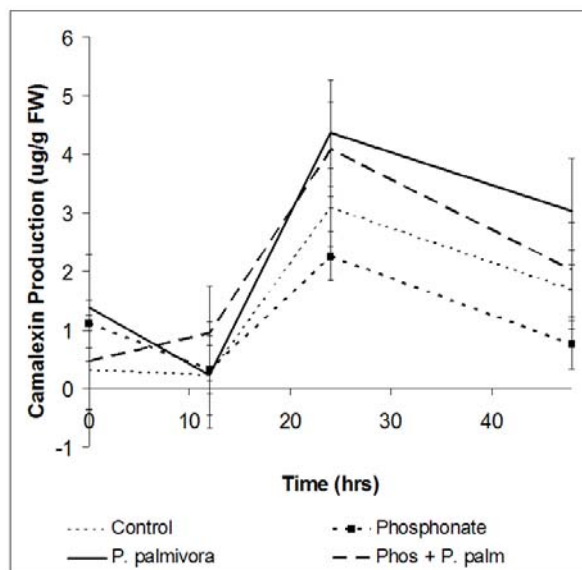


Figure 1. Camalexin Production in *Arabidopsis* treated with phosphonate and infected with *P. palmivora*; n=6; bars indicate standard error

Accumulation as a result of abiotic factors reported previously (3), may explain why camalexin was routinely detected in uninoculated, untreated seedlings indicating that experimental conditions were enough to cause a small amount of camalexin to accumulate.

Phosphonate reduced camalexin accumulation in uninoculated seedlings at 24 and 48 h pi to levels below that of controls. Inoculation of phosphonate-treated seedlings restored camalexin accumulation however differences were not significant. This reduction in camalexin accumulation following phosphonate application was unexpected but has been confirmed in subsequent experiments.

Although phosphonate has been shown to elicit an incompatible interaction between *Arabidopsis* and *P. palmivora* (2), camalexin does not appear to play a significant role in phosphonate-induced resistance. This indicates that the interaction between phosphonate-treated *Arabidopsis* and *P. palmivora* involves the activation of multiple lines of defence. The signalling pathways activated by phosphonate in this pathosystem still remain unclear, but the availability of *Arabidopsis* defence-related mutants will aid elucidation of the interaction.

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79 Characterisation of *Phytophthora capsici* isolates from black pepper in Vietnam

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INTRODUCTION

Since the establishment of *Phytophthora capsici* as the causal agent of black pepper foot rot in Vietnam (Truong *et al.* 2008), there is an urgent need to understand the population structure of this pathogen. The role of genetic diversity and geographic structuring of *P. capsici* foot rot epidemics of black pepper is not known. It is assumed that environmental effects, host susceptibility and cultivation practices facilitate selection pressures, which in turn affects the changes in the pathogen population structure, and subsequently the pattern of disease incidence. In order to make decisions regarding the direction of disease management strategies, the population structure of this pathogen needs to be explored. We begin to address these issues by testing two hypotheses. The first is that only one mating type exists in the *P. capsici* population from black pepper in Vietnam. The second is that the *P. capsici* population is genetically undifferentiated in two different climatic regions.

MATERIALS AND METHODS

Isolates origin. *Phytophthora capsici* was isolated from soil and plant samples obtained from provinces in the Southeast region (SE) and the North Central Coast region (NCC) (Truong *et al.* 2008).

Mating type analysis. Each isolate was paired on V8 Agar with known A1 and A2 testers. Test isolates producing oospores with both A1 and A2 were scored as A1A2. The test was replicated 3 times.

DNA extraction, RAMS and REP-PCR protocol and genetic analysis. Fungal mycelium was grown in liquid medium, incubated at 25°C in the dark for 5–6 days and DNA was extracted. The RAMS and REP-PCR fingerprinting protocols were performed as previously described by Hantula *et al.* (1996) and Rademaker & Bruijn (1997). Cluster analysis was performed using the DICE similarity coefficient and UPGMA agglomeration in the program NTSYSpc.

RESULTS

Mating type analysis. Both A1 and A2 mating types were found to co-exist within the same farm in 13 cases in Dong Nai (SE) and Quang Tri (NCC) provinces. In addition, A1 and A2 mating types were also observed to co-exist on the same plant in one case in Quang Tri province.

RAM and REP analysis. In order to assess the overall genetic diversity of the whole population and relationship between the two regional subpopulations, the combined data from RAMS and REP analyses were used to construct an UPGMA dendrogram (Fig 1). The *P. capsici* isolates from black pepper are distributed in two main groups, I and II, which are differentiated at DICE similarity of 53%. Group I comprises 114 isolates with 108 belonging to one large clonal group, two isolates in a small clonal group and four with unique phenotypes. Group II comprises four isolates, all with unique phenotypes. The genetic similarity analysis showed that more than 91% of all isolates were genetically identical and the whole population was nearly homogeneous. The clustering of isolates in the dendrogram does not correlate with geographic origin. The large clonal group

consists of isolates obtained from all provinces in both regions. The results also indicate that isolates are not genetically correlated with mating type.

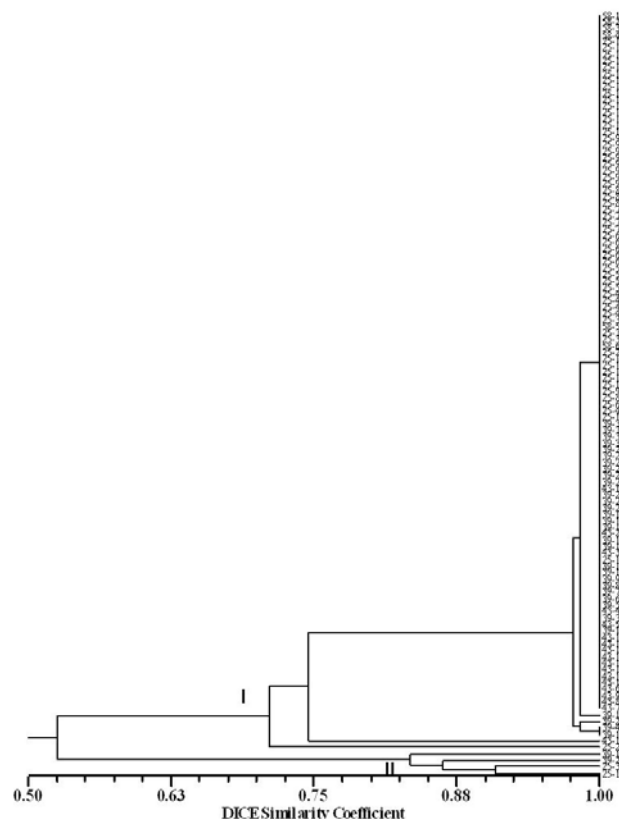


Figure 1. UPGMA dendrograms of 118 isolates of *Phytophthora capsici* based on combined RAMS and REP data

DISCUSSION

The analysis of *P. capsici* isolates obtained from various growing regions revealed the presence of both mating types in two different climatic regions, with the A2 type detected at higher frequency than the A1 type. Overall the level of genetic diversity detected among the *P. capsici* isolates from black pepper was relatively low. One hundred and eight isolates were found to be identical in their RAMS and REP phenotypes. The genetic pattern of the *P. capsici* population was not found to be associated with geographic origin, and hence the two regional subpopulations were undifferentiated. The current report provides preliminary but useful information concerning the extent of genetic diversity and geographic distribution of *P. capsici* from black pepper in Vietnam.

ACKNOWLEDGEMENTS

The authors wish to thank ACIAR for sponsoring this research.

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80 Characterisation of *Phytophthora capsici* Isolates from chilli in Vietnam

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INTRODUCTION

Phytophthora capsici, a pathogen of a range of tropical crops, such as black pepper, betel, cacao and rubber, is not managed effectively in developing countries due to lack of knowledge on the interactions between the pathogen and its many hosts (Drenth and Guest 2004). Although diseases of crops caused by *P. capsici* in temperate regions have been investigated in great detail, a lot less is known about this species in the South East Asian region. *P. capsici* is the causal agent of black pepper foot rot. With the need for integrated disease management in mind, questions arise as to the cross-infectivity of the pathogen from different hosts and whether chilli could be an alternative host harbouring the black pepper pathogen. This study described the testing of three hypotheses. The first was that *P. capsici* isolates from chilli were pathogenic on black pepper and conversely black pepper isolates on chilli. The second was that chilli isolates were genetically different from black pepper isolates. The third was that there was genetic diversity among *P. capsici* isolates from chilli.

MATERIALS AND METHODS

Phytophthora capsici isolates. Twenty-two isolates of *P. capsici* from chilli pepper (*Capsicum frutescens* L.) recovered from soil and diseased plants in Da Lat and Quang Nam provinces were used in this study. Twenty-four isolates of *P. capsici* from black pepper were also used to compare with isolates from chilli pepper in the genetic analysis. In addition, an isolate each representing *Phytophthora* species *P. palmivora* and *P. nicotianae* was also included as species comparison in the RAMS and REP-PCR analysis.

Pathogenicity. Six leaves of black pepper or chilli were placed in a metal tray in 3 rows on layers of moist tissue. Leaves of black pepper or chilli were pricked with a sterile needle and inoculated with 5 µL of zoospore suspension. Uninoculated samples were similarly inoculated with sterilised deionised water.

DNA extraction, RAMS and REP-PCR protocol and genetic analysis. Fungal mycelium was grown in liquid medium, incubated at 25°C in the dark for 5–6 days and DNA was extracted. The RAMS and REP-PCR fingerprinting protocols were performed as previously described by Hantula *et al.* (1996) and Rademaker & Bruijn (1997). Cluster analysis was performed using the DICE similarity coefficient and UPGMA agglomeration in the program NTSYSpc.

RESULTS

Pathogenicity test. Chilli leaves inoculated with isolates from black pepper developed 'water-soaked lesions', whereas the isolates from chilli tested on black pepper leaves showed lesions with fimbriate margins.

RAM and REP analysis. The data from RAMS and REP-PCR were combined to construct an UPGMA dendrogram (Figure. 1). A total of 7 phenotypes were detected, of which 4 were unique and 3 represented clonal groups. The first clonal group comprised 4 chilli isolates from Dalat. The second contained 18 chilli isolates from Quang Nam. The third was 20 isolates from black pepper. The four unique isolates were from black pepper.

The isolates from black pepper were separated from the chilli isolates at a similarity level of <50%, both groups clearly distinct from *P. palmivora* and *P. nicotianae*.

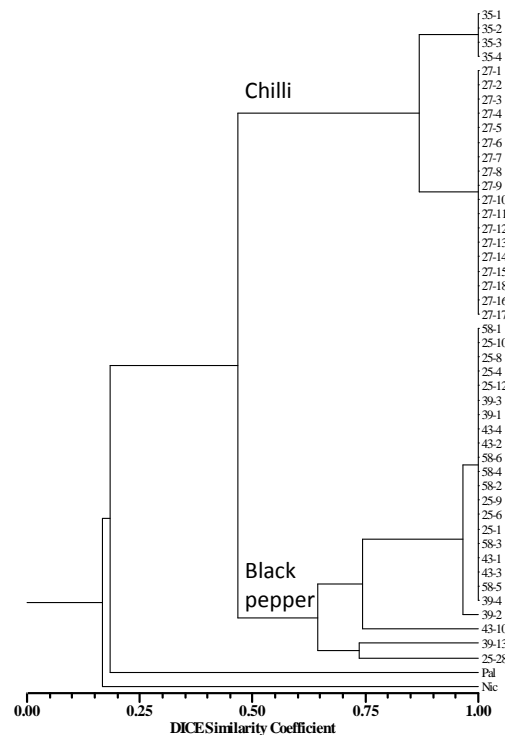


Figure 1. UPGMA dendrograms of 46 isolates *P. capsici* from chilli and black pepper including references, *P. palmivora* (Pal) and *P. nicotianae* (Nic) based on RAMS and REP data.

DISCUSSION

The isolates from chilli were highly pathogenic to black pepper and isolates from black pepper were also virulent to chilli but disease symptoms were slightly different. The result indicated that the *P. capsici* population in Vietnam infects both crops, but are genetically differentiated. This may pose a serious threat to black pepper production in Vietnam in the long term as the pathogen population is potentially able to generate and maintain high levels of genetic diversity. Further studies should be investigated to elucidate the interaction between *P. capsici* population and its alternative hosts. This has an important significance for the integrated disease management to diseases on the black pepper and other crops caused by this species.

ACKNOWLEDGEMENTS

The authors wish to thank ACIAR for sponsoring this research.

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53 The inhibitory effect of sumac stem extract on some fungal plant pathogens

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INTRODUCTION

Plants produce more than 10000 secondary metabolites with low molecular weights, many of which prevent plant infections to diseases and pests (2). Methanolic extract of sumac rich in hydrolysable tannins and proanthocyanidins (5) has demonstrated high inhibitory activity to *Bacillus*, *Staphylococcus aureus* and *Enterobacter phlei* (6).

MATERIALS AND METHODS

Plant material. Sumac (*Rhus coriaria*) stems at the final stage of growth were collected from their natural habitat in Hamedan, Iran, in September 2007. The stems were dried at ambient temperature in shade at a laboratory. They were ground using a mill, and passed through a 1-mm mesh metal sieve.

Extraction method. Soluble compounds were extracted in methanol (1). Five grams of powdered stem was placed into a bottle containing 100 ml absolute methanol. The bottles were capped and shaken in a rotary shaker at 350 rpm at 20°C for 24 hours. Then, 75 ml of the solution from each bottle was removed and poured separately into another bottle. To each bottle was added 25 ml of sterilised distilled water and 100 ml hexane. The bottles were capped and shaken at 350 rpm for 2 hours. Subsequently, the aqueous phase was separated. The methanolic extracts were placed into a hood to evaporate the methanol.

Pathogens. *Phytophthora drechsleri* and *Rhizoctonia solani* were isolated from beet roots, and *Fusarium oxysporum* and *Bipolaris sorokiniana* were isolated from chickpea and wheat roots, respectively. The pathogenicity of the pathogens confirmed on their hosts.

Poison food technique. Based on the method of Hagerman and Butler (4), the inhibitory effect of 500, 1000, 1500, and 2000 ppm from the extract was examined using a completely randomised design with four replicates. The zero level (just solvent) was used as control. The calculated extract for each concentration required to poison 100 ml PDA was dissolved in 1.5 ml of methanol, and added to the autoclaved PDA when cooled down to 40°C. The poisoned PDA was dispensed in 25 ml aliquots into 9 cm Petri plates and allowed to cool. The plates were inoculated with 6 mm diameter discs from cultures of the fungi. The inoculated plates were incubated at 25°C. Colony diameters were measured frequently until the fungal growth in the control plates completely covered the plates. The experiment was repeated twice.

Calculation of inhibitory percentage. The inhibitory effect of different extract concentrations was calculated using the following formula (3):

$$IP = 100(C-T)/C$$

where IP is inhibitory percentage; C is the colony diameter of the control; and T is the colony diameter of the treatment.

RESULTS AND DISCUSSION

The inhibitory effects of extracts increased as the extract concentration increased. The effectiveness of the extract against *R. solani* and *B. sorokiniana* was more than against *F. oxysporum* and *P. drechsleri* (Table 1). Complete inhibition of growth of *R. solani* took place at 1500 ppm, while 2000 ppm of extract had 81% inhibitory effect on *B. sorokiniana*. It is likely that by increasing extract concentration against this and the other two fungal species, the inhibitory effect could be increased to 100%.

Table 1. Growth inhibition (%) of four fungi by different concentrations (ppm) of methanolic extract from sumac.

Extract conc	<i>F. oxysporum</i>	<i>R. solani</i>	<i>P. drechsleri</i>	<i>B. sorokiniana</i>
500	28.12	84.46	1.08	68.56
1000	48.04	89.64	1.90	72.15
1500	51.01	100	8.15	74.26
2000	59.08	100	59.51	81.22

The crude methanolic extract and isolated constituents of another member of this genus, *Rhus glabra*, was effective against 11 gram negative and positive bacteria (7) and nine pathogenic fungi (6). The research result was in conformity with previous findings regarding antifungal activity of sumac extract. In conclusion, the crude methanolic extract of sumac can be used as an antifungal agent against the examined fungi, particularly *R. solani* and *B. sorokiniana*.

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33 Aerial photography—a tool to monitor Mallee onion stunt

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INTRODUCTION

Mallee onion stunt (MOS) is a widespread disease that was identified in the Murray Mallee of South Australia in 2005 (1). It is associated with specific strains of *Rhizoctonia solani* which cause stunted patches of economic significance. *Rhizoctonia* bare patch (AG 8) is a common disease of both cereals and onions. Cereal crops are often rotated with onions. Monitoring this disease over large areas is time consuming and complex. In this research aerial imagery was evaluated as a tool for MOS mapping and monitoring.

MATERIALS AND METHODS

High resolution aerial imagery was captured using two digital cameras mounted on an unmanned aerial vehicle (UAV). Normalised Difference Vegetation Index (NDVI = Red-NIR/Red+NIR) was used to estimate relative biomass. Colour images of complete onion pivots were constructed by mosaicing multiple images. Images and maps were geo-referenced using DGPS ground control points. Low NDVI areas were identified as putative disease patches, and paired soil samples were collected from within the disease patches and in adjacent normal areas. DNA was extracted from soil and assayed for *Rhizoctonia* using PCR (2).

RESULTS AND DISCUSSION

MOS patches are shown in an example colour image of an onion pivot (Fig. 1), and an NDVI (relative biomass) map from the same pivot is shown in Fig. 2. An example of a mosaiced complete pivot image is shown in Fig. 3. Levels of *Rhizoctonia* DNA (AG 8) in soil from stunted patches were significantly higher (396.4 pg DNA/g soil) than those from adjacent normal patches (26.1). Aerial imagery using a UAV is a rapid, cheap and effective tool for monitoring and mapping onion diseases. Raw images were viewed on a field computer within 15 minutes of commencement of the UAV flight. These were used to identify general areas of interest for immediate disease scouting, and geo-referenced NDVI maps allowed sampling points to be located within 1m on the ground. The imagery was particularly useful and interesting for growers, who were often able to contribute valuable interpretation of biomass patterns. Further research to collect more data from sampling points identified from biomass maps is planned.



Figure 1. Colour image showing MOS bare patches.

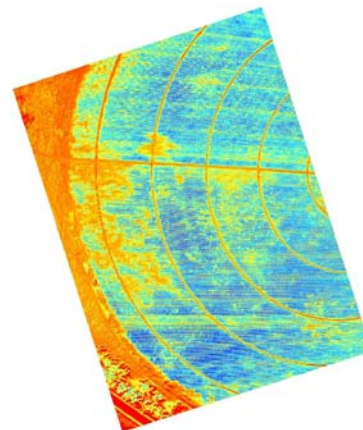


Figure 2. NDVI (relative biomass) map. MOS patches (low biomass) are light tone patches.



Figure 3. Complete mosaiced onion pivot image.

ACKNOWLEDGEMENTS

This project was facilitated by Horticulture Australia Limited in partnership with AUSVEG and was funded by the Onion Levy. The Australian Government provides matched funding for all HAL Research and Development activities, and onion growers in the Mallee region are thanked for their support. Thank you to the SARDI Diagnostic laboratory for PCR assays, and thank you to Angela Lush for laboratory support.

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81 Survey of viruses infecting sweet potato crops in New Zealand

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INTRODUCTION

Sweet potato or kumara (*Ipomoea batatas*) is important culturally and as a food source in New Zealand. The annual production of about 20,000 tonnes is grown mainly in the districts of Auckland, Bay of Plenty and Kaipara in the North Island. In January 2008, virus symptoms that included chlorotic spots, ring spots, vein clearing and mottling were observed on the leaves of commercial sweet potato crops (mainly cvs. Beauregard, Owairaka Red and Toka Toka Gold) growing in the three main production areas. A survey was done to determine the extent of virus infection affecting these crops.

MATERIALS AND METHODS

Samples. Fifty to 100 leaves were collected randomly from each of 26 different fields, five in Pukekohe (Auckland), nine in Gisborne (Bay of Plenty) and twelve in Dargaville (Kaipara). Leaves from each field were bulked into groups of 10, giving a total of 173 composite samples.

Real-time PCR. Total nucleic acid was extracted from all 173 composite samples, and used in real-time PCR assays specific for *Sweet potato leaf curl virus* (SPLCV) and real-time reverse transcription (RT)-PCR assays specific for *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus G* (SPVG), and *Sweet potato virus 2* (SPV2; synonym *Sweet potato virus Y*).

Graft inoculation. Representative plants from each field were grafted onto 3–4 week old *Ipomoea setosa* plants. Symptoms were monitored for 3–5 weeks and leaves were collected for testing.

NCM-ELISA. Nitrocellulose membrane enzyme-linked immunosorbent assays (International Potato Center-CIP, Lima, Peru) were done on the original sweet potato samples and grafted *I. setosa*. The following viruses were tested for: *Cucumber mosaic virus* (CMV), *C-6 virus*, *Sweet potato caulimovirus-like virus* (SPCaLV), *Sweet potato chlorotic fleck virus* (SPCFV), SPCSV, *Sweet potato latent virus* (SwPLV) and *Sweet potato mild specking virus* (SPMSV).

RESULTS AND DISCUSSION

Real-time RT-PCR detected the potyviruses SPVG, SPFMV and SPV2 in many of the samples. Table 1 presents results showing these three viruses are common in the three areas surveyed. Figure 1 shows symptoms in two different cultivars infected with the three viruses. SPFMV and SPVG have been reported in New Zealand (1, 2) but SPV2 had not been reported previously (3). Samples infected with SPV2, were found as single infections, in co-infection with SPVG and SPFMV, or with SPVG but not SPFMV, but no samples were co-infected with SPV2 and SPFMV when SPVG was absent. No samples were infected with SPLCV.

None of the original kumara samples were positive for CMV, C-6 virus, SPCSV, SPCaLV, SPCFV, SwPLV or SPMSV by NCM-ELISA. No samples tested positive for SPCSV by real-time PCR or NCM-ELISA.

Symptoms on grafted *I. setosa* were typical of potyvirus infection and no additional viruses were detected when they were tested by NCM-ELISA.

Table 1. Viruses in sweet potato in New Zealand.

	No. of fields tested (No. of samples tested)	No. of infected fields (No. of infected samples)		
		SPV2	SPFMV	SPVG
Dargaville	12 (103)	6 (35)	12 (70)	11(82)
Pukekohe	5 (40)	4 (17)	2 (12)	5 (27)
Gisborne	9 (30)	7 (18)	9 (30)	9 (30)
Total	26 (173)	17(70)	23(112)	25(139)



Figure 1. Two sweet potato cultivars showing A) chlorotic spots and B) ring spots. Both samples were infected with SPVG, SPFMV and SPV2.

Single potyvirus infections cause mild or no symptoms in sweet potato, and consequently yield is not significantly reduced. However, co-infection with other viruses such as SPCSV produces a synergistic effect and more severe disease symptoms (4).

SPCaLV, SPCFV, SwPLV have been reported in New Zealand previously (1) but they were not detected during this survey.

ACKNOWLEDGMENT

We would like to thank the Kumara growers who supported this work by allowing us to sample their crops, and without whose co-operation this survey would not have been possible.

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54 Evaluation of commercial cultivars for control of white blister rust in *Brassica rapa* and *Brassica oleracea* vegetables

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INTRODUCTION

White blister rust of Brassicaceae is caused by an oomycete *Albugo candida*. The disease affects many economically important crops including *Brassica oleracea* and *B. rapa* vegetables in Australia and around the world (Petkowski 2008). White or creamy pustules filled with zoosporeangia on leaves, stems and heads as well as hypertrophy and hyperplasia of plant organs downgrade the aesthetics and marketability of the produce. In vegetable production, white blister rust is commonly controlled with fungicides. Planting resistant cultivars, however, is the most cost-effective and desirable disease control method (Li et al 2007). A number of commercial cultivars of *B. oleracea* and *B. rapa* are sold as "tolerant" to *A. candida* pathotypes occurring on these species in Australia.

We report on the evaluation of commercial cvs of *B. oleracea* and *B. rapa* in glasshouse screening trials.

MATERIALS AND METHODS

In two glasshouse experiments, seedlings of 10 cvs of *B. rapa* and 12 cvs of *B. oleracea* were tested for resistance to *A. candida* collections from Chinese cabbage and broccoli, respectively. Each experiment included hosts with reported susceptibility or resistance as controls. In each experiment, seeds were sown in seed-raising mix in plastic multipot trays of 144 pots per tray. Seedlings were irrigated twice a day for 1 minute by overhead sprinklers. Plants in both experiments were fertilised weekly with Aquasol™.

Inocula were prepared by suspending zoosporeangia in sterile distilled water at the concentration of 1×10^5 zoosporeangia per mL. Prior to inoculation, the suspensions were incubated for four hours at 13 °C to induce zoospore release. Inocula were applied twice in each of the experiments using a trigger atomiser on seedlings previously misted with sterile distilled water. The first inoculation was at the fully developed cotyledon stage and the second at the first true leaf growth stage. Seedlings were covered with plastic sheets after each inoculation for 24 hours to ensure sufficient leaf wetness for infection. White blister incidence and severity on hosts was assessed on 4 week-old and on 5 week-old seedlings in experiments with *B. rapa* and *B. oleracea*, respectively. Incidence was expressed as a percentage of the seedlings with symptoms. Disease severity was rated on seedlings with sori using a 0–4 scale. Experiments were designed as randomised complete blocks of treatments (12 hosts) with 8 replications. Data were analysed using ANOVA.

RESULTS AND DISCUSSION

All varieties of *B. rapa* vegetables tested were either very or moderately susceptible to white blister (Table 1). Miyako and Walz were significantly more susceptible than other cultivars tested. Pak choi cv Seven Gates had smaller blisters surrounded by discoloured rings of leaf tissues, indicating some level of resistance to the *A. candida* collection tested.

Table 1. Incidence and severity of white blister rust on seedlings of commercial cultivars of *B. rapa* and *B. oleracea* vegetables inoculated with *A. candida* collections from Chinese cabbage and broccoli in

glasshouse conditions. Means followed by different letters are significantly different at the 5% level.

B. rapa Cultivar n=96	Mean Incidence (%)	Mean Severity Scale (0–4)	B. oleracea Cultivar n=96	Mean Incidence (%)	Mean Severity Scale (0–4)
Miyako	91.3 ^a	2.2 ^a	Greenbelt*	79.8 ^a	1.6 ^a
Walz	80.1 ^b	1.3 ^b	Forte*	76.6 ^a	1.7 ^a
Mei Qoing	72.9 ^b	1.3 ^b	Highfield	53.6 ^b	1.1 ^b
Cv 001	70.6 ^b	1.1 ^b	Brittany	44.5 ^{bc}	0.9 ^b
Matilda	66.9 ^b	1.0 ^b	Millenium	35.7 ^{cd}	0.6 ^{bc}
Seven Gates	60.5 ^b	0.9 ^b	Summer Love	35.6 ^{cd}	0.8 ^{bc}
Manoko	60.4 ^b	0.8 ^b	Sting	25.3 ^d	0.3 ^c
Reward*	58.0 ^b	1.0 ^b	Cyrus	4.2 ^e	0.1 ^c
Torch*	55.3 ^b	1.4 ^b	Avalanche	0.0	0.0
Joi Choi	56.3 ^b	0.7 ^b	Abacus	0.0	0.0
Kailaan*	5.6 ^c	0.0 ^c	Romulus	0.0	0.0
Regent*	0.0 ^c	0.0 ^c	NIZ17-1091	0.0	0.0

*) Cultivars incorporated either as positive or negative controls. Cultivars Regent (*B. napus*) and Kailaan (*B. oleracea*) are negative controls.

Brussels sprouts cultivars Abacus and Romulus had no white blister, cv Cyrus had a very low incidence (4.2%) of seedlings with blisters and cv Millenium was susceptible (36% of seedling affected). All but one cauliflower cultivar, Avalanche, were susceptible (Table 1). Cabbage cv Sting, which is susceptible to European *A. candida*, was moderately susceptible to the Australian *A. candida*. The moderately susceptible cv NIZ17-1091, however, was resistant to the collection tested. White blister rust was the most severe on cauliflower cv Forte followed by broccoli Greenbelt. White blister rust control can be improved by planting less susceptible cvs combined with fungicide sprays timed by using the *Brassica_{spot}*™ disease risk predictive model (Petkowski 2008).

ACKNOWLEDGEMENTS

The authors thank seed companies for supplying seeds and HAL, AusVeg, the State Government of Victoria and the Federal Government for financial support.

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34 Diatrypaceae species associated with grapevines and other hosts in New South Wales

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INTRODUCTION

The fungus *Eutypa lata* is responsible for the canker disease of grapevines known as Eutypa dieback. Entry of the fungus via pruning wounds and the formation of cankers in the vascular tissue of grapevines results in a slow decline and dieback that reduces vigour, yield and vineyard productivity (1). Symptoms may include stunted shoots with shortened internodes, and a characteristic wedge shaped lesion in the trunks and canes of infected vines. In addition to *E. lata*, a number of other species belonging to the Diatrypaceae can be found on grapevines and other hosts in and around vineyards. To date, the role of many of the diatrypaceous species in grapevine decline is unknown. The incidence and distribution of species in the Diatrypaceae was documented during a recent survey of vineyards throughout New South Wales (NSW).

MATERIALS AND METHODS

Surveys were conducted from 73 vineyards throughout NSW between November 2006 and April 2008 to study fungi associated with grapevine trunk diseases. Wood samples were taken from 1789 grapevines displaying foliar symptoms typical of Eutypa dieback or evidence of dead spurs, cankers, or bleached and discoloured tissue. Isolations of fungi were made by directly plating out pieces of diseased tissue onto PDA after surface sterilisation in 1% sodium hypochlorite. Samples were incubated at 25°C and monitored for the appearance of fungi. Diatrypaceous species were tentatively identified based on gross cultural morphology. In December 2008, additional surveys and collections were conducted from grapevines and other hosts, both in the Hunter Valley and Tumbarumba. Isolations of diatrypaceous fungi from these samples were made directly from ascospores, with species tentatively identified based on morphology of the teleomorph and confirmed via sequencing and analysis of ribosomal DNA regions.

RESULTS AND DISCUSSION

Morphological and molecular analyses of fungi isolated from diseased wood and cultured from fruiting bodies revealed the presence of five species belonging to the Diatrypaceae. In addition to *E. lata*, *Eutypa leptoplaca*, *Cryptovalsa ampelina* and species of both *Diatrypella* and *Eutypella* were isolated. In total, 79 isolates were collected, 12 of *E. lata*, 23 of *C. ampelina*, 1 isolate of *E. leptoplaca*, 19 of *Eutypella* and 24 of *Diatrypella* (Table 1), although isolates from the latter genera could not be identified to species.

These surveys have shown that Eutypa dieback is more widespread than first thought and may be increasing in prominence, especially in cooler climates where lower temperatures and high rainfall favour the growth of *E. lata* (2). Additionally, several other species within the Diatrypaceae are present not only on grapevines, but on a number of other hosts in and around vineyards. Many of these species are widely distributed in NSW and have been shown to have wider geographic ranges and occur in greater numbers than *E. lata*.

While *E. lata*, and the lesser-known *C. ampelina* are recognised pathogens of grapevines (3), little is known about the other

species that are commonly found both on grapevines and other hosts. Additional studies are required to determine the pathogenicity of the diatrypaceous species on grapevines. The impact of these species and their role in grapevine decline is under investigation.

Table 1. Incidence and distribution of Diatrypaceous fungi associated with grapevines and other hosts in New South Wales.

Region	Host	Fungi
Big Rivers	<i>Vitis vinifera</i>	<i>Diatrypella</i>
		<i>Eutypa lata</i>
		<i>Cryptovalsa ampelina</i>
Central Ranges	<i>Vitis vinifera</i>	<i>Eutypella</i>
		<i>Diatrypella</i>
		<i>E. lata</i>
		<i>C. ampelina</i>
		<i>Eutypella</i>
Southern NSW	<i>Vitis vinifera</i>	<i>Diatrypella</i>
		<i>E. lata</i>
		<i>C. ampelina</i>
		<i>Eutypa leptoplaca</i>
		<i>C. ampelina</i>
		<i>Populus nigra-italica</i>
		<i>Ulmus procera</i>
		<i>C. ampelina</i>
		<i>Eutypella</i>
		<i>Diatrypella</i>
Northern Rivers	<i>Vitis vinifera</i>	<i>C. ampelina</i>
		<i>Eutypella</i>
Northern Slopes	<i>Vitis vinifera</i>	<i>Diatrypella</i>
		<i>Eutypella</i>
Hunter Valley	<i>Vitis vinifera</i>	<i>C. ampelina</i>
		<i>Eutypella</i>
		<i>Diatrypella</i>
		<i>Citrus paradise</i>
		<i>Eutypella</i>
		<i>Diatrypella</i>
		<i>Citrus sinensis</i>
<i>Ficus carica</i>		
	<i>Fraxinus excelsior</i>	<i>Diatrypella</i>

ACKNOWLEDGEMENTS

This work was supported by the Winegrowing Futures Program, a joint initiative of the Grape and Wine Research and Development Corporation and the National Wine and Grape Industry Centre. The authors wish to thank Florent Trouillas (UC Davis) and Mark Sosnowski (SARDI) for technical assistance.

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13 Biological control of *Uncinula necator* by mycophagous mites

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INTRODUCTION

A number of abiotic and biotic factors influence the incidence, severity, and spatial scale of diseases in natural and managed plant systems (2, 3). Although the function of such factors as temperature, humidity, and host plant resistance traits have been relatively well-studied, the impact of natural enemies on interactions between plants and pathogens has received less attention. Plant pathologists have investigated various microorganisms as potential biological control agents of microbial plant pathogens (1, 8). The ability of arthropods to regulate plant pathogens is poorly understood. Leaf-inhabiting mites that are thought to feed on fungi (mycophagous mites) and other microbes are extremely common on many woody perennial plant species (9, 10) and represent a potentially significant group of natural enemies of fungal plant pathogens that attack leaves and fruit. *Erysiphe necator*, the causal agent of grape powdery mildew, is a worldwide pathogen (6). The disease imposes considerable damages to grapes by reduction of the quality and quantity of the product in Sistan region. The powdery mildew agent has a great capacity to develop resistance to synthetic fungicides (4). Therefore, it seems that biological control is the best control approach of the disease.

The objective of the project was to identify mycophagous mites that inhabit grape leaves infected and uninfected by *Erysiphe necator*.

METHODS AND RESULTS

Plenty of mites were collected from grape leaves infected and uninfected by *Erysiphe necator*. A faunistic survey was conducted in August 2007 to collect and identify mites living on the grapevine cultivar Yaghoti in Sistan region, Iran. Six species belonging to six families were collected and identified from infected leaves (Table 1). Two species were collected and identified from uninfected leaves (Table 1).

population dynamics (8). English-Loeb *et al.* (5) have suggested that *Orthotydeus lambi* (Acari: Tydeidae) could be useful as a biological control agent of powdery mildew on cultivated grapes under vineyard conditions. *Erysiphe necator* is obligately parasitic, and with the exception of absorptive haustoria within epidermal cells of the host, the body of the pathogen resides on the plant surface (6). This characteristic makes it vulnerable to grazing by mycophagous mites.

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Mite species	Mite diet	Presence on leaves	
		Infected	Uninfected
<i>Lasioseius mcgregori</i> (Ascidae)	Predatory/ mycophagous	+	
<i>Typhlodromus sp.</i> (Phytoseiidae)	Predatory/ mycophagous	+	+
<i>Tyrophagus putrescentiae</i> Schrank (Acaridae)	Mycophagous	+	+
<i>Rhizoglyphus robini</i> (Acaridae)	Mycophagous	+	
<i>Anystis baccharum</i> (Anystidae)	Predatory/ mycophagous	+	
<i>Tydeus sp.</i> (Tydeidae)	Predatory/ mycophagous	+	

DISCUSSION

The experiment revealed that the population of mycophagous mites in grape leaves infected with *E. necator* was significantly higher than uninfected leaves. It is well recognised that some arthropods including mites use fungi as a food resource, although it is less understood how this influences fungal

35 First report of a eucalypt yellowing disease in Syria and its similarity to Mundulla yellows

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INTRODUCTION

Eucalyptus camaldulensis is widely grown in Syria for land rehabilitation, amenity, and as roadside trees. Seed was imported repeatedly on a large scale from different locations in Australia up to the 1970s (I. Nahal, L. Makki pers comm). This has resulted in a national population of *E. camaldulensis* including both subspecies *camaldulensis* and *obtusa*, as well as local hybrids of the two. Around 500 000 eucalypt seedlings are being produced annually from Syrian seed by 11 government nurseries for distribution around the different regions.

During a visit to ICARDA by D. Hanold in October 2008, yellowing symptoms similar to the Mundulla Yellows (MY) syndrome (1) were noted. The MY-like symptoms appeared to be widespread in some locations, and preliminary surveys of several sites were carried out to obtain further information about its distribution. While a witches' broom disease without associated yellowing symptoms had been described earlier from Syrian eucalypts (2), this is the first report of a eucalypt yellowing disease from that country.

MATERIALS AND METHODS

Based on the description of MY distribution in Australia (1), trees not adjacent to roads as well as roadside trees were characterised. Survey sites in the regions around Aleppo, Tel Hadya, Ein Dara, Idleb, Tabaqah, along the road to the coast west of Homs, and along the Damascus-Aleppo highway were mapped (Fig. 1).

Photographs were taken of trees at each site and additional information recorded as available. Individual trees on the ICARDA station (Tel Hadya) were also numbered for future reference and leaf samples were taken to Adelaide and stored frozen for molecular analysis. Symptomatic seedlings identified in a nursery were planted at ICARDA together with asymptomatic controls to observe disease progress.

RESULTS

Eucalypts with symptoms resembling the descriptors of MY (Table 1) and including interveinal chlorosis, epicormic shoots and asymmetric yellowing of the crown were observed in all areas except west of Homs where only asymptomatic eucalypts were observed. *E. camaldulensis* both in single and mixed species plantings on roadsides, in paddocks, soil rehabilitation areas, seed gardens and nurseries were affected. Symptoms occurred on single trees adjacent to asymptomatic ones, in clusters, and as disease gradients similar to the distribution of MY in Australia (1).

DISCUSSION

MY is defined by characteristic descriptors distinguishing it from yellowing disorders due to environmental factors. Its cause is unknown, but a biotic, contagious agent has been implicated (1). Yellowing diseases of eucalypts closely resembling the Australian MY have also been reported from Spain and South America (1). This is the first report of a similar syndrome from Syria and the Middle East.

Syria has developed a large, isolated population of *E. camaldulensis* over the last century. Little insect or fungal damage has been observed suggesting that many pathogens affecting the species in Australia are absent. Nevertheless MY-like symptoms virtually identical to the syndrome in Australia are present. Further epidemiological and phenotypic, as well as molecular comparison of Syrian and Australian symptomatic *E. camaldulensis* is needed and may provide information on aetiology and possibly origin of the disease.



Figure 1. Map of Syria.

Table 1. Comparison of the Syrian eucalypt yellowing disease (SEY) with descriptors of MY (1).

Descriptors	SEY	MY
Leaf symptoms:		
interveinal chlorosis	+	+
distortion of leaf margins	+	+
necrotic pin spots	+	+
no dead leaves on twigs	+	+
Epicormic shoots	+	+
Twig dieback	+	+
Disease stages:		
early (yellow patches in foliage)	+	+
medium (yellow epicormic shoots)	+	+
late (overall tree dieback)	+	+
Affected trees next to healthy ones	+	+
Trees of all ages affected	+	+
No recovery observed	+	+
Not cured by pruning	+	+
Symptoms in paddocks and roadsides	+	+

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of I. Nahal and B. Bayaa, Aleppo University; L. Makki, Syrian Department of Agriculture; A. El-Ahmed, R. Brettell, S. Kumari and the staff and management of ICARDA.

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36 New host records for '*Candidatus Phytoplasma aurantifolia*' in Australia

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INTRODUCTION

Phytoplasmas are unculturable wall-less prokaryotes within the class *Mollicutes* that infect plant phloem vessels and are transmitted by phloem feeding insects (2). They are implicated in diseases of a wide range of plant species. Symptoms of phytoplasma infection include reduced leaf size 'little leaf', yellowing of leaves, proliferation of stems leaves and flowers (witches' broom) and floral abnormalities (1, 3).

Plant health surveys were carried out by the Australian Quarantine and Inspection Service (AQIS) in northern Western Australia (northern WA) and the Northern Territory (NT) from 2006 to 2008. This paper reports on the new host records of phytoplasma collected during these surveys.

MATERIALS AND METHODS

Plants exhibiting symptoms of phytoplasma infection (little leaf or witches' broom) during plant health surveys in northern WA and the NT from 2006 to 2008 were collected for diagnostic confirmation. Plant parts with symptomatic new growth were collected into plastic bags and kept cool. Within 2 days of collection leaf petioles and midribs were excised from the symptomatic material, dehydrated over anhydrous CaCl₂ and stored at 4°C where possible.

Samples were forwarded to Bioscience North Australia, Charles Darwin University for molecular analysis. Sample analysis included polymerase chain reaction (PCR) assays using universal phytoplasma specific primers. Strain analysis was performed by restriction fragment length polymorphism (JR153, KN11) or by sequencing (JR428, JR429).

RESULTS AND DISCUSSION

'*Candidatus Phytoplasma aurantifolia*' is reported for the first time associated with disease of *Ipomea aquatica*, *Jatropha gossypifolia*, *Ocimum basilicum* and *Canavalia rosea* (Table 1). These new records were all detected in WA whilst no new records were detected in the NT during this time. These phytoplasma records represent a diverse host range.

'*Ca. .P. aurantifolia*' has a wide host range across a diverse group of plant families and is widespread throughout Australia. The suggested Australasian/Asian origin of this phytoplasma may in part explain its success in harsh environments and wide host range (1, 3).

All new records were obtained in northern WA which has a particularly harsh climate. During surveillance it was observed that some species of annual plants infected with phytoplasma do not produce seed and remain green for longer than the same uninfected hosts (Ray, personal observation). Perhaps the occurrence of a diverse host range for '*Ca. .P. aurantifolia*' is a survival mechanism for both the phytoplasma and the leaf hopper vectors by providing live host material during periods when other hosts have died, the creation of a green bridge.

Table 1. Details of new host records for '*Ca. .P. aurantifolia*' in Australia.

Species, Common name	Location	Strain	Coll. No.
<i>Ipomea aquatica</i> , Kangkung	Broome, WA	TBB	JR153
<i>Jatropha gossypifolia</i> , Bellyache bush	Cable Beach, WA	SPLL-V4	JR428
<i>Ocimum basilicum</i> , Lemon basil	Wattle Downs, WA	TBB	JR429
<i>Canavalia rosea</i> , Beach bean	Lombadina Beach, WA	SPLL-V4	KN11

ACKNOWLEDGEMENTS

Thanks to Karen Gibb, Anna Padovan and Claire Streten of Bioscience North Australia, Charles Darwin University for diagnostics. Andrew Mitchell and Kirsty Neaylon (AQIS) are gratefully acknowledged for some collections, and A. Mitchell for identifying plant specimens.

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37 A comparative study of methods for screening chickpea and wheat for resistance to root-lesion nematode *Pratylenchus thornei*

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²© State of Queensland, Department of Employment, Economic Development and Innovation, 2009

INTRODUCTION

Several quantitative methods are available for testing resistance of crops to root-lesion nematode (*Pratylenchus thornei*) under controlled environments. This study aimed to compare growth times for wheat and chickpea cultivars and the nematode extraction procedure of Whitehead tray, (Whitehead and Hemming 1965) with shake-elution (Moore *et al* 1992) and misting (Hooper 1986) methods. The objective was to optimise differences between susceptible and resistant chickpea lines for plant breeding purposes.

MATERIAL AND METHODS

Five chickpea and two wheat lines covering a range of resistance to *P. thornei* were selected for testing. The design consisted of 3 replicates in randomised blocks with 6 harvest times and 3 extraction methods. For each time x variety x replicate combination there were 2 pots to enable nematode comparison from one half of each pot using standard Whitehead tray method, while roots were extracted from the other half for either shake-elution or misting. Single plants were grown in pots of 330 g of steam-sterilised vertosol maintained between 22–25°C in a glasshouse on a 2 cm tension bottom-watering system. A 15 ml suspension to provide 10,000 *P. thornei*/kg soil was pipetted around the seed at planting. At each harvest of 12, 14, 16, 18 and 20 weeks, soil with roots from pots was sectioned longitudinally into halves for nematode extractions. For all 3 extraction procedures room temperatures were in the range of 22–26°C and nematodes were collected on a 20 µm sieve. Whitehead and shake-elution extractions were assessed at 1, 2, 3, 4 and 7 days while misting extractions were assessed at 4 and 7 days. Nematodes were counted in a 1-mL Hawksley slide and expressed as number of *P. thornei*/kg soil (oven-dry equivalent) or *P. thornei*/g root (fresh weight). A multi-factorial data analysis was performed using $\ln(x+c)$ where x = nematodes/kg soil and c = constant.

RESULTS AND DISCUSSION.

The Whitehead tray method extracted significantly ($P < 0.001$) more *P. thornei* than either misting or shake-elution (Fig. 1 a and b). Growing chickpeas for a longer period (18–20 weeks) than wheat (16–18 weeks) gave maximum discrimination of resistance/susceptibility in cultivars (Fig. 2). The extraction efficiency for 2 days was 70% of that at 7 days when using Whitehead trays. All the above results showed similar differences between treatments whether expressed as *P. thornei*/kg soil or as *P. thornei*/g root. The Whitehead trays were found to be less labour intensive than misting and shake-elution procedures, and more practical for assessing large numbers of plants for resistance.

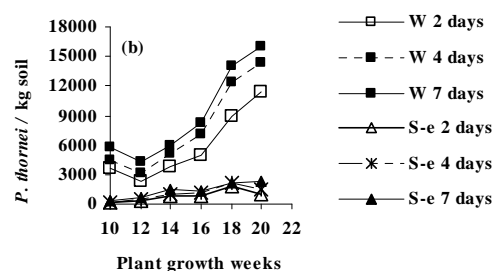
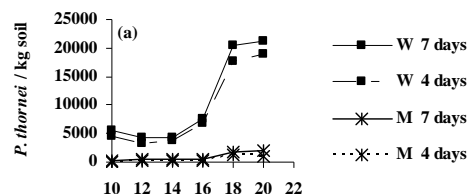


Figure 1. For each harvest the total number of *P. thornei* extracted from wheat and chickpea with Whitehead trays (W) was significantly higher ($P < 0.001$) than misting (M; Fig. 1a) and shake-elution (S-e; Fig. 1b).

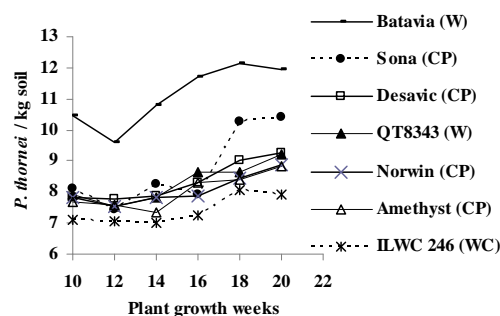


Figure 2. Longer growth periods allowed better discrimination among chickpea cultivars. Value of l.s.d. at 18 weeks and 20 weeks ($P = 0.05$) = 0.82 and 1.32 respectively. W=wheat, CP=chickpea, WC=wild chickpea.

ACKNOWLEDGEMENTS

Kerry Bell for statistical analysis and Indooroopilly Research laboratories for use of their misting facilities.

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82 Multi-locus sequence typing of isolates of *Pseudomonas syringae* pv. *actinidiae*, a biosecurity risk pathogen

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INTRODUCTION

Pseudomonas syringae pv. *actinidiae* (*P.s.a.*) is a bacterium that was first recorded in Japan (1), causing a trunk canker on kiwifruit (*Actinidia deliciosa* 'Hayward'). This disease has not been recorded on kiwifruit in New Zealand. PCR primers were designed to detect this pathogen to prevent importation into New Zealand on germplasm. To prove specificity of these primers isolates from different geographic locations were tested.

MATERIALS AND METHODS

Cultures. Cultures for DNA extraction were sourced from the International Collection of Micro-organisms from Plants (ICMP), New Zealand; Korean Agricultural Culture Collection (KACC), Republic of Korea; National Institute of Agrobiological Sciences (NIAS), Japan; National Collection of Plant Pathogenic Bacteria (NCPBB), UK; and the Culture Collection of Plant Pathogenic Bacteria (PD), The Netherlands.

PCR detection. Primers were designed and tested against 20 strains of *P.s.a.* from international culture collections. Six strains were not detected by these primers (Table 1), and multi-locus sequence typing (MLST) was conducted to study these strains in more detail. Sequence was included of three bacteria found on kiwifruit orchards (2), *Pseudomonas* sp., *P. fluorescens* (*P.f.*) and *P. syringae* (*P.s.*) and four representatives from Group 1 (3), *P.s. maculicola*, *P.s. tomato*, *P.s. theae*, and *P.s.a.* *P. s.* is in Group 2, and *P. f.* is an outgroup.

Multi-locus sequence typing. Five housekeeping genes were sequenced: those encoding sigma factor 70, aconitate hydratase B, citrate synthase, phosphoglucosyltransferase, and gyrase. The 16S–23S rDNA intergenic spacer (IGS) region was also sequenced.

Phylogenetic analysis. Analyses were performed on individual gene sequences as well as on the concatenated data set using maximum parsimony.

RESULTS

By BLAST analysis, the sequence of the IGS region of KAAC 10660 was most similar to *Rahnella aquatilis*, an unrelated saprotroph. Isolates KAAC 10582, ISPAVE-B-020, ISPAVE-B-019, PD2766 and PD2774 were most similar to *P. syringae*, and the type strain Kw-11 to *P.s.a.* DNA from KAAC 10660 was not amplified by any MLST primers. All MLST gene phylogenies yielded similar results: PD2766 was most similar to *P. f.*, KAAC 10582 to *Pseudomonas* sp., ISPAVE-B-020, ISPAVE-B-019 and PD2774 to *P.s. syringae*. None of the isolates not detected by PCR primers F1/R2 and F3/R4 was closely associated with the type strain Kw-11, or other Group 1 pathovars.

DISCUSSION

These results indicate that isolate KAAC 10660 is an unrelated saprotroph that has been misidentified. Because the other five sequenced atypical isolates were not genetically related to Group 1 pathovars of *P. syringae*, these are also misidentifications.

Table 1. Isolates of *Pseudomonas syringae* pv. *actinidiae* detected by primer sets F1/R2 and F3/R4

Isolate name	Collection	Origin	Detected
Kw-11	ICMP	Japan	+
Kw-1		Japan	+
Kw-30		Japan	+
Kw-41		Japan	+
KAAC 10582	KAAC	Korea	-
KAAC 10584		Korea	+
KAAC 10594		Korea	+
KAAC 10659		Japan	+
KAAC 10660		Korea	-
KAAC 10754		Korea	+
FTRS L1	NIAS	Japan	+
Sar1		Japan	+
Sar2		Japan	+
Kiw4		Japan	+
Wa1		Japan	+
Wa2		Japan	+
ISPAVE-B-020	NCPBB	Italy	-
ISPAVE-B-019		Italy	-
PD 2766	PD	USA	-
PD 2774		USA	-

ACKNOWLEDGEMENTS

This work was funded by FfrST contract CO2X0501. DNA was provided by L. Liefing, and advice by R. Newcomb.

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95 DNA barcoding to support biosecurity decisions

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INTRODUCTION

Precise pathogen identifications in the biosecurity context must be accurate and timely, as identification delays can affect trade, as well as response efforts to invasive exotic species. The need to rapidly identify cryptic fungi by traditional morphological techniques presents a distinct challenge to plant diagnostic laboratories. The use of DNA barcoding techniques to identify morphologically cryptic species addresses this challenge and has gained momentum recently, with the establishment of the International Consortium for the Barcode of Life.

The MAFBNZ Plant Health and Environment Laboratory (PHEL) is currently developing a DNA barcoding platform to resolve diagnostic cases for which other methods are unable to provide timely and accurate results. An example of the usefulness of this tool is presented here.

The genus *Diaporthe* includes plant pathogens, plant endophytes and species associated with dying and dead vegetation that are usually observed as their *Phomopsis* anamorphs. These fungi are associated with disease symptoms on a wide range of species, including many economically important plants. Our objective was to develop capability to utilise barcoding to precisely and quickly identify fungal species, in order to assist in the determination of the regulatory status of intercepted organisms, and to allow more informed biosecurity decisions.

METHODS

Isolates from surveillance of fungi on plants in New Zealand and all strains deposited as either *Diaporthe* or *Phomopsis* from the International Collection of Microorganisms (ICMP) were included. A portion of the mycelia for all samples was stored in 20% glycerol at minus 80°C and DNA for sequencing was extracted from the remaining mycelia. Sequences of the ITS region were generated using primer pair ITS1/ITS4 (1). The ITS sequences of authoritatively identified *Diaporthe* and *Phomopsis* strains were selected from recent revisionary studies. Sequences were stored and analysed with the software program Geneious (Biomatters Ltd, Auckland, New Zealand)

RESULTS

To date, the *Diaporthe/Phomopsis* barcode database consists of 22 sequences from PHEL, 68 New Zealand strains from ICMP culture collection, and 92 from reference strains (Fig. 1). Continued survey work and addition of strains from other collections will expand and contribute new branches to the tree.

The barcode database has proven to be a powerful tool to rapidly and accurately identify unknown strains. For instance, strains from surveillance (PHEL09-2009-2132) and the culture collection (ICMP2141) were identified to species level (Fig. 1), providing an initial identification in the first case, and clarification in the second.

Isolates without a species name in this project (e.g. Groups A-E) will be confirmed by further taxonomic work.

DISCUSSION

Development of a DNA barcoding platform can lead to resolution of many questions, including rapid determination if certain species, including undescribed taxa, are present in a country.

However, the barcoding approach could challenge or conflict with phytosanitary regulation. For instance some isolates were found consistently mis-identified at species and higher taxonomic levels, and these organisms could be new to the country.

DNA barcoding will likely be an international standard for plant pathogen identification, with the caveat that this powerful tool relies upon sequences generated from well-characterised voucher specimens (preferably including the type species) in order for DNA barcode results to be well supported.

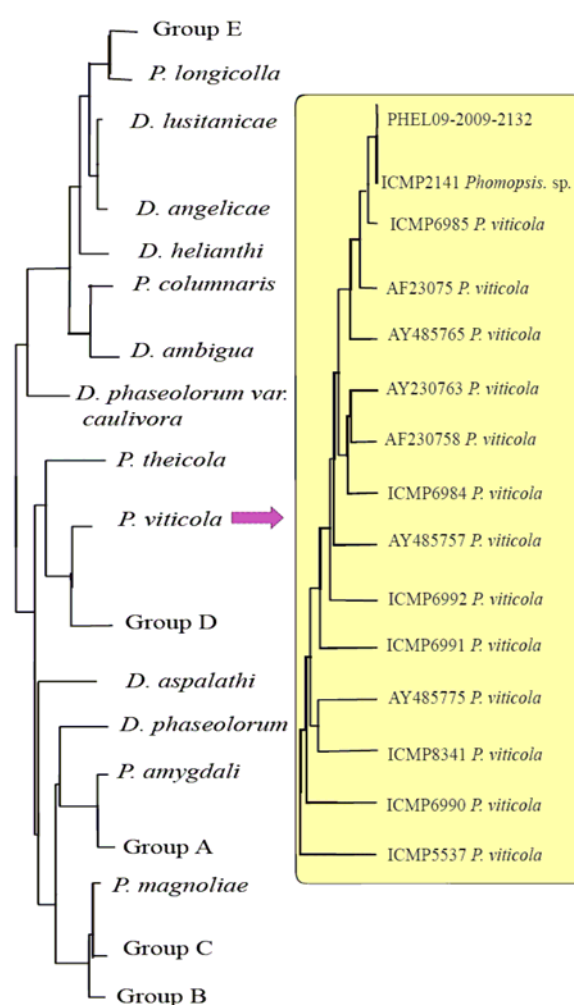


Figure 1. Phylogenetic tree of *Diaporthe* and *Phomopsis* derived from Neighbour-joining analysis. Expanded data set of *P. viticola* clade is presented in more detail.

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14 In vitro screening of potential antagonists of *Xanthomonas translucens* infecting pistachio

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INTRODUCTION

X. translucens is the causal agent of dieback disease of pistachio. The bacterium infects the vascular tissues of the trees, causing discolouration of the xylem, lesions on the trunk and major limbs, decline and, in some cases, death (1). Although hygiene and application of quaternary ammonium disinfectant to pruning wounds have been recommended to limit the spread of the disease (2), effective control methods are lacking. Biological control offers potential in managing this disease. The aim of this research is to assess the ability of bacteria to antagonise *X. translucens* *in vitro*, and thus explore the potential of biological control in managing pistachio dieback.

MATERIALS AND METHODS

Pathogen. Isolate KI of *X. translucens* (*Xtp_KI*), obtained from a commercial orchard in Kyalite, NSW was used (3). The isolate was resuscitated from storage in sucrose peptone broth (SPB) and glycerol at -80°C.

Potential antagonists. Eight isolates of bacteria obtained from pistachio wood were tested, along with one isolate of *Bacillus subtilis*. Seven of the pistachio isolates were courtesy of E. Facelli and C. Taylor.

Preliminary screening. A protocol to assess the antagonistic ability of the above bacteria was modified from Parente *et al.* (4). One hundred µl of a suspension of 10⁶ CFU/ml of *Xtp_KI* was spread on either sucrose peptone agar (SPA) or nutrient agar (NA). After drying, a 6-mm diameter well was punched into the agar, aseptically, and filled with 20 µl of 3 day-old culture of the chosen antagonist. At this stage, the concentration of the antagonist suspension was not determined. Each antagonist treatment was replicated five times and sterile distilled water was used for controls. Antagonism was assessed by measuring the inhibition zone around the wells 48 hours after treatment.

Antibiotic activity of the antagonists. Production of diffusible antibiotics inhibitory to *Xtp_KI* was investigated using a procedure modified from Mari *et al.* (5). The bacterial isolates were cultured in SPB, at 28°C on a rotary shaker. After 48 hours, the suspensions were centrifuged at 10,000g for 20 minutes and the supernatants filtered through 0.45 µm membrane filters. Following the protocol described above, 20 µl of culture filtrate was placed into each well. Antibiotic activity was assessed by measuring inhibition zones around wells 48 hours after treatment.

RESULTS AND DISCUSSION

One isolate (64161-7) efficiently inhibited the growth of *Xtp_KI*, both on SPA and NA, with clear inhibition zones of >10mm radius. Four isolates (SUPP, PC397, PC506 and PC507) caused moderate inhibition of growth of *Xtp_KI* on SPA and NA (inhibition zones <10mm). *B. subtilis* was moderately effective on SPA only, whereas isolates CBP and 71164-16 produced inhibition zones of <5mm on NA only. Therefore, the ability of the bacteria to inhibit growth of *Xtp_KI* *in vitro* was influenced by the culture medium used.

Although most of the bacterial isolates inhibited *Xtp_KI* in dual culture, there was no evidence of antibiotic activity in culture filtrates. *B. subtilis* was expected to produce diffusible antibiotics, but most reports concern antifungal activity. Methods for assessing production of diffusible antibiotics are being refined. Selected isolates are being tested for ability to colonise pistachio wood and to reduce colonisation of the wood by *Xtp_KI*.

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83 Uniform distribution of powdery mildew conidia using an improved spore settling tower

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INTRODUCTION

Powdery mildew of cucurbits (*Podosphaera fusca* (Fr.) S. Blumer) is an obligate parasite, unable to be cultured and maintained on agar media. Thereby, it provides a challenge to researchers studying the infection processes in the laboratory. More problematic is that the pathogen is sensitive to free water. Water damages conidia by negatively affecting viability and infectivity (Sivapalan, 1993). Thus, conidia cannot be applied to plants using water suspensions. Use of dry conidia applied by dusting or blowing from infected leaves onto test plants is commonly used in artificially inoculating plants. This approach allows for deposition of conidia onto plant surfaces, but with little uniformity of distribution (Reifschneider and Boiteux, 1988). Techniques such as the use of paint brushes and cotton swabs provide improved uniformity of conidial distribution, but are lacking in convenience and accuracy. A spore-settling tower applying Stoke's law of sedimentation as proposed by Reifschneider and Boiteux (1988) has provided a more convenient and repeatable method for inoculation of powdery mildews. These authors constructed a plywood tower and demonstrated successful use of a low vacuum induced air inrush to dislodge conidia from infected leaves, and to disperse them effectively over test plants. This design was improved by the authors to enhance useability for inoculation of powdery mildews onto test plants.

MATERIALS AND METHODS

The spore settling tower was constructed from a flanged Perspex cylinder (100 cm in height, 50 cm diameter, 20 mm thick). The complete tower comprised of the cylinder, a top cover, and a base plate. The top cover incorporates a vacuum line and air valve, a vacuum gauge, a small removable lid with a 25mm inlet valve leading to an internal inoculum platform suspended below. The vacuum line and air valve is used to connect the tower to a vacuum pump, and the internal vacuum applied is measured by the attached gauge. The inlet valve in the removable lid is used to sharply break the vacuum inside the tower dislodging conidia from the inoculum source (freshly harvested infected leaves). The internal inoculum platform (12 cm diameter) was constructed under the top cover with three holes, each with a 9.0 cm diameter in its side walls (20 cm deep) to allow inoculum to be expelled and shower the test plants placed on the base plate at the bottom of the tower. The whole construction was designed and fabricated to maintain an internal vacuum by the use of rubber gaskets and sealants.

The distribution of conidia dispersed through the operation of the spore settling tower was examined using water agar (2%) trap plates. The base of the trap plates were marked with a 1.0 cm grid using a marker pen. Three open trap plates were used as replicates per inoculation run, and arranged on the base plate of the spore settling tower. Powdery mildew (*P. fusca*) maintained on cucurbit plants in the glasshouse, was used as the inoculum source. Leaves were cut into disks 2.0 cm² in diameter. In each inoculation run, 20 leaf disks (~ 2.5 g f.w.) were placed in an open Petri plate and covered with a layer of tape-fastened open plastic mesh (1 cm² pore size). The inoculum was placed on the inoculum platform, the water agar trap plates placed on the

base plate and the unit sealed. A vacuum of 20 kPa (taking ~ 10 sec) was applied followed by closing of the air valve. Sudden opening of the inlet valve in the removable lid resulted in a sharp break in the vacuum causing a sudden inrush air onto the inoculum source and dislodgment of conidia. The trap plates at the base of the unit plate were then exposed to the resulting shower of conidia. The unit was left undisturbed for a minimum of 120 seconds after breaking of vacuum to optimise conidial distribution (Reifschneider and Boiteux, 1988). After each inoculation run, the media in the water agar trap plates was cut into 1 cm squares and fifteen randomly selected sections placed on glass microscope slides, stained with lactophenol cotton blue and observed under a light microscope to evaluate the distribution of conidia by counting. The data were analysed using a statistical analysis system (SAS).

RESULTS AND DISCUSSION

The spore settling tower developed in this study is relatively easy to operate for inoculation of plants with powdery mildew pathogens. The transparent Perspex construction allows users to monitor the process inside the tower. Provision of a vacuum gauge allows for more consistent conditions to be achieved, resulting in higher repeatability among inoculation runs. The incorporation of an air inlet valve (not present in the unit designed by Reifschneider and Boiteux, 1988) allows for easy breaking of vacuum, while modifications to the inoculum platform further improved useability.

The distribution of conidia observed in this study was uniform with no significant difference between the numbers observed within and between trap plates $P < 0.05$. The results showed that each trap plate received an average of 27 ± 2 conidia / cm². The results of this study agreed with those of Reifschneider and Boiteux (1988), who determined that a settling tower was a more practical method, and ensured improved uniformity in the distribution of conidia on test plants than dusting techniques using paint brushes.

ACKNOWLEDGEMENTS

Brett Jahnke, technical officer, School of Land, Crop and Food Sciences, The University of Queensland for engineering and construction of the spore settling tower.

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84 The effect of high nutrient loads on disease severity due to *Phytophthora cinnamomi* in urban bushland

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INTRODUCTION

The soilborne pathogen *Phytophthora cinnamomi* has become infamous for its destruction of native Australian vegetation communities, particularly in Western Australia and Victoria. More recently, the pathogen has been reported to occur widely around NSW. *Phytophthora* has been indicted with dieback of iconic species including *Angophora costata*, *Eucalyptus botryoides*, *E. piperita* and *Corymbia gummifera* around Sydney Harbour. However, bushland reserves in which these trees occur are also often subject to high nutrient loads from urban runoff. Environmental abiotic factors can play a major role in either enhancing or suppressing the disease severity on a susceptible host. Seedlings of *E. maculata* and *E. pilularis* grown with lower amounts of nitrogen and phosphorus express more severe disease symptoms (1). Conversely, disease severity increased with increasing levels of inorganic, but not organic, nitrogen application in durian and papaya inoculated with *P. palmivora* (2).

This study investigates the relationship between soil nutrient loads and the severity of disease due to *Phytophthora* in four tree species found in bushland and parks around Sydney Harbour.

MATERIALS AND METHODS

Site selection. Four 20 x 20 m quadrats were set up in the Lawry Plunkett Reserve in Mosman, NSW. Sites were identified based on drainage runoff and the level of disturbance (invasive weeds) (Table 1). Ten soil samples were taken from each of the four sites for analysis. Samples were collected from the root zone of *A. costata*, *E. botryoides*, *E. piperita* and *C. gummifera*.

Table 1. Sites in the Lawry Plunkett Reserve from which soil was sampled for analysis of nutrient levels, microbial activity and *Phytophthora*.

Site	Description
1	Highly disturbed, <i>Phytophthora</i> isolated
2	Highly disturbed, <i>Phytophthora</i> isolated
3	Moderately disturbed, <i>Phytophthora</i> isolated
4	Moderately disturbed, <i>Phytophthora</i> isolated

Soil analysis. All 40 soil samples were analysed for the presence of *Phytophthora* by lupin baiting (3). The concentration of nitrogen (N), phosphorus (P) and other minerals were determined for three of the 10 samples taken from each site. Microbial activity was measured for all soil samples using the FDA assay (4).

Glasshouse trials. The effect of N on the severity of disease due to *P. cinnamomi* is being assessed in a glasshouse trial. *A. costata*, *E. piperita*, *E. botryoides*, *C. gummifera* and *Pinus radiata* (susceptible control) seedlings were inoculated with *P. cinnamomi* are treated with 0 mg/ml and 100 mg/mL ammonium nitrate every two weeks. Plant health is being assessed over time, and root and shoot weight will be measured at the end of the experiment.

RESULTS AND DISCUSSION

The occurrence of *Phytophthora*. *P. cinnamomi* was isolated from all four sites sampled. A second species of *Phytophthora* was isolated from Sites 1 and 2 and is being identified.

Nutrient analysis. The nutrient levels varied significantly within and between sites. Site 2 had higher levels of total soil N (due to higher levels of NH_4^+) than the other three sites (Fig 1). A drainage channel may account for the high N load observed in soil sampled from Site 2. Soil P levels were not significantly different between sites. Magnesium levels were highest in soil samples from Site 4. Aluminium was present at extreme levels at Site 3. The effect of excess nitrogen on disease severity in the four tree species is currently being assessed in a glasshouse study.

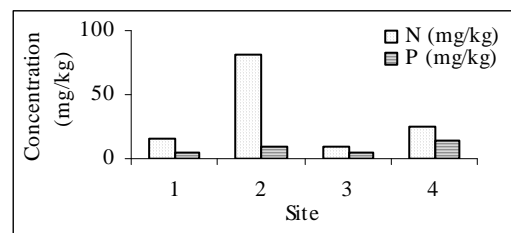


Figure 1. Levels of soil nitrogen and phosphorus at the four sites in the Lawry Plunkett Reserve.

Microbial activity. At $3.5 \mu\text{g FDA g}^{-1}\text{min}^{-1}$, microbial activity was greatest in Site 4, although this was not statistically significant ($p = 0.11$). Site 3 had the lowest microbial activity at $2.2 \mu\text{g FDA g}^{-1}\text{min}^{-1}$ (Figure 2).

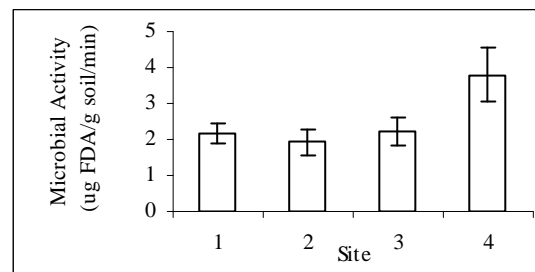


Figure 2. Microbial activity at the four sites in the Lawry Plunkett Reserve as determined by the FDA assay.

In urban areas, nutrients available in the soil can fluctuate due to run off. High amounts of run off resulting in soil nutrient loading may play a significant role in either the severity or suppression of dieback disease associated with *P. cinnamomi*. A greater understanding of the interaction between dieback and nutrient loads will enable effective management decisions for urban areas where nutrient loads are usually prominent and *Phytophthora* is present.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of Mosman Council in the supply of seedlings and the soil analysis in this study.

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15 Characterisation of the causal agent of pistachio dieback as a new pathovar of *Xanthomonas translucens*, *X. translucens* pv. *pistaciae* pv. nov.

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INTRODUCTION

Pistachio dieback has limited the expansion of the pistachio industry in Australia over the past 15 years. It is caused by two genetically distinct groups of strains of *X. translucens* (1, 2). However, the pistachio pathogen is atypical because it has a dicotyledonous woody host, in contrast to typical *X. translucens* that cause disease in monocotyledonous hosts in the *Poaceae*. Also, the characterisation of integrons, which are known to have played a key role in genetic diversification of *Xanthomonas*, suggested that the pistachio pathogen represented a new pathovar of the species (3). Here, we report use of DNA-DNA hybridisation and *gyrB* gene sequencing to further clarify the taxonomic position of the pistachio pathogen and establish its phylogeny among other *Xanthomonas*.

MATERIALS AND METHODS

DNA-DNA hybridisation. DNA/DNA hybridisation analyses were conducted to determine the DNA similarity of the reference strains of the two groups of the pistachio pathogen (ICMP 16316 and ICMP 16317) (2) to the pathotype strains of three *X. translucens* pathovars, namely *X. translucens* pv. *translucens* (LMG 876), *X. translucens* pv. *poae* (LMG 728) and *X. translucens* pv. *graminis* (LMG 726). The type strains of *X. theicola* (LMG 8764) and *X. hyacinthi* (LMG 739) were included as outgroups. Each hybridisation was conducted two-four times.

***gyrB* phylogeny.** DNA extraction, PCR and sequence analysis of the *gyrB* gene were performed as described by Parkinson *et al.* (4). Sequences were determined for the reference strains ICMP 16316 and ICMP 16317 of the pistachio pathogen, and compared to that of other *Xanthomonas* available in the database (4).

RESULTS

DNA-DNA hybridisation. DNA-DNA hybridisation between the two strains of the pistachio pathogen was 84%. When compared to *X. translucens* pathovars, one group had the highest homology with *X. translucens* pv. *poae*, with 84% hybridisation, whereas the other group had the highest homology with *X. translucens* pv. *graminis*, with 90% hybridisation. Hybridisation values of both pistachio strains with *X. theicola* and *X. hyacinthi* averaged no more than 54%.

***gyrB* phylogeny.** In sequence alignments, the two groups of the pistachio pathogen showed 96% homology. When compared with other *Xanthomonas*, the highest similarity was to pathovars of *X. translucens*, with percentages ranging from 95 to 99%, whereas the similarity to other *Xanthomonas* species and their pathovars ranged from 81 to 86%. The two exceptions were *X. theicola* and *X. hyacinthi*, which showed similarity values of 92 and 93%, respectively, both with strains of the two groups. In the phylogenetic tree, both groups clustered among *X. translucens* pathovars, but as distinct lineages.

DISCUSSION

DNA-DNA hybridisation is considered to provide definitive species-level identification: strains from the same species have above 70% homology, whereas strains from different species show homology values averaging 40–50%. Therefore, our results confirmed the classification of the pistachio pathogen as an *X. translucens*.

The clustering of the pistachio pathogen among *X. translucens* pathovars in the *gyrB* phylogeny further confirms the DNA-DNA hybridisation results and suggests that the pistachio pathogen has originated through host switching of one of the *Xanthomonas* ancestors that had a monocotyledonous host.

These results, together with the distinct pathogenicity to pistachio and the consistent discrimination of the pistachio pathogen from other *X. translucens* (2, 3), support its designation as a new pathovar of the species, for which we propose the name *Xanthomonas translucens* pv. *pistaciae* pv. nov.

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38 Interactions between *Leptosphaeria maculans* and fungi associated with canola stubble

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INTRODUCTION

Blackleg, caused by *Leptosphaeria maculans*, is of major economic importance in the canola-growing areas of Australia. The pathogen survives on stubble and releases ascospores from pseudothecia.

Antagonistic activity of fungi against *L. maculans* has been documented (1, 2). Antagonism, competition, stimulation of growth and decomposition of the stubble may affect survival and sporulation of *L. maculans*. A better understanding of the interactions between *L. maculans* and potentially antagonistic fungi on canola stubble could facilitate the development of strategies to reduce inoculum of the pathogen and contribute to the control of blackleg.

MATERIALS AND METHODS

In total, 35 species of fungi, including *L. maculans*, were isolated from canola stubble or associated soil collected in South Australia.

Dual culture on agar plates. *L. maculans* and potential antagonists were co-inoculated in triplicate on potato dextrose agar (PDA) in Petri dishes and incubated at room temperature for up to 3 weeks. Changes in colony appearance, hyphal growth and sporulation of *L. maculans* were examined.

Dual culture on agar-coated slides. Three replicate water agar-coated slides were inoculated for each test species and incubated at room temperature for up to 2 weeks. Interactions between hyphae of *L. maculans* and each test fungus were examined microscopically at 4-day intervals.

Inoculation of blackleg-affected stubble. Blackleg-affected stubble, three segments on moist sand in each of four Petri dishes, was inoculated with each test species and incubated at 15°C in a 12 h photoperiod. After 6 weeks, the density of pseudothecia (number in a 0.5 × 1 cm area) was assessed for each stubble segment. Germination of ascospores collected from stubble inoculated with each test fungus was assessed.

Effect of fungi on decay of canola stubble. Disease-free stubble inoculated with *Stachybotrys chartarum* and *Coprinus* sp. was incubated at 20°C in a 12 h photoperiod for 2 months and weight loss determined.

Statistical analysis. Data were subjected to ANOVA.

RESULTS

Macroscopic and microscopic observations of plates and agar-coated slides, respectively, showed lysis, deformation, overgrowth of hyphae and inhibition of growth and sporulation of *L. maculans* by *Alternaria* spp., *Arthrobotrys* sp., *Aspergillus* sp., *Fusarium equiseti*, *Gliocladium roseum*, *Myrothecium* sp., *Trichoderma aureoviride*, *Sordaria* sp., *S. chartarum* and an unknown Coelomycete.

None of the 21 species tested eliminated *L. maculans* from stubble. Density of pseudothecia of *L. maculans* on stubble was

reduced by over 67% compared with pathogen-alone controls following inoculation with *F. equiseti*, *G. roseum*, *T. aureoviride*, *Sordaria* sp. or the Coelomycete. Over 70% of *L. maculans* ascospores obtained from the stubble germinated after 24 h at 20°C, irrespective of the co-inoculated fungus.

The mass of canola stubble inoculated with *S. chartarum* and *Coprinus* sp. was reduced almost 2-fold compared with that of uninoculated controls (Table 1).

Table 1. The effect of *Coprinus* sp. and *Stachybotrys chartarum* on weight of canola stubble after 2 months at 20°C.

Test fungus	Mean decrease of stubble weight (mg) ¹	
	Fresh weight	Air-dry weight
Control ²	5.1	12.1
<i>Coprinus</i> sp.	9.6	20.1
<i>Stachybotrys chartarum</i>	13.4	26.3

¹ Mean of the difference of weight before inoculation and after incubation.

² Each control stubble segment received 2 ml sterile distilled water, whereas each treated stubble segment was inoculated with two plugs of PDA culture of *Coprinus* sp. (plus 2 ml of sterile distilled water) or 2 ml of spore suspension of *S. chartarum*.

DISCUSSION

This study provides information on interactions between *L. maculans* and a wide range of fungal species, isolated from canola stubble or associated soil, on agar media and on canola stubble. Several stubble-associated fungi both antagonised the pathogen *in vitro* and reduced pseudothecium formation on canola stubble. In particular, *Coprinus* sp. and *S. chartarum*, as antagonists of *L. maculans* and effective decomposers of canola stubble in this study, have potential to reduce pathogen inoculum on stubble in the field. This warrants further investigation, including longer-term field studies, to assess the suitability of fungal antagonists as a biological means of controlling blackleg.

ACKNOWLEDGEMENTS

We thank T. Potter, SARDI, for supplying stubble.

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86 First report of tomato yellow leaf curl virus in pepper (*Capsicum annuum*) fields in Iran

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INTRODUCTION

Tomato yellow leaf curl virus (TYLCV) is one of the most devastating pathogens affecting tomato (*Solanum lycopersicum*) worldwide and is very important in Iran. TYLCV is transmitted by *Bemisia tabaci* in persistent and circulative manner. Tomato is the most important host for TYLCV. Symptoms on hosts except pepper include stunting, yellowing, leaf curl and flower senescence, whereas no distinct symptoms has been reported on pepper and those which occasionally observed, are caused by vector feeding (4). There are conflicting reports regarding the susceptibility of peppers (*Capsicum* spp.) to TYLCV and in Spain, *C. annuum* was reported as a host of an uncharacterised strain of TYLCV (5).

MATERIALS AND METHODS

In order to detection of TYLCV in Southern Iran, samples were collected from tomato and pepper fields in Bandar Abbas region including Rezvan and Sarkhun areas and jiroft region in 2007. Attention to disease symptoms including stunting, leaf curling, yellowing and deformation of stem end, 38 samples from Sarkhun, 80 samples from Rezvan and 10 samples from jiroft were collected. DNA extraction was performed with Dellaporta protocol (2). Virus detection was conducted by PCR with specific primers (3) and also by DAS-ELISA (1).

RESULTS AND DISCUSSION

After DNA extraction and specific PCR, 2 samples of pepper and 6 samples of tomato from 11 samples of pepper and 27 samples of tomato in Sarkhun, 68 samples from 80 samples of tomato in Rezvan and 4 samples of tomato and 2 samples of pepper from 4 samples of tomato and 6 samples of pepper in jiroft were infected by PCR (Table 1), while DAS-ELISA couldn't detect any infection. Indeed a viral DNA fragment of 670 bp including a part of coat protein and movement protein genes was amplified in positive samples (Figure 1). Infection of 85% of Rezvan samples and about 21% of Sarkhun samples and 60% of jiroft samples indicate the most incidence of TYLCV in Rezvan area and also detection of virus in pepper samples indicate presence of TYLCV in pepper fields in southern Iran. This is the first report of pepper plants infected by *Tomato yellow leaf curl virus* in Iran.

Table 1. sampling regions and number of collected and positive samples

Regions	Collected samples (Tomato)	Collected samples (Pepper)	Positive samples (Tomato)	Positive samples (Pepper)
Rezvan	80	0	69	0
Sarkhun	25	13	5	4
Jiroft	6	4	4	2

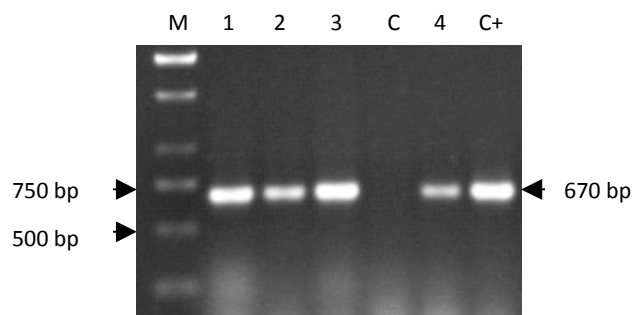


Figure 1. A viral DNA fragment of 670 bp was amplified in positive samples.

(M): 1 Kb ladder- (1,2,3): Tomato positive samples-(C-): Negative control- (4): Pepper positive sample and (C+): positive control

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39 Seed-borne concerns with wheat streak mosaic virus in 2008

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INTRODUCTION

Wheat streak mosaic virus (WSMV), is a sap-borne viral disease transmitted by the wheat curl mite (WCM). Western Australian research reported globally for the first time in 2005 that low levels of seed transmission (<1%) of WSMV can occur in wheat (1).

When WSMV is introduced through seed-borne infections its distribution is expected to be as scattered individual plants, with leaf streaking and stunting, across the area sown with infected seed. However, the relative importance of seed-borne dispersal of WSMV to its epidemiology has not been confirmed in the field. We now report data that suggests that seedborne transmission may be contributing to substantial yield loss in some crops.

MATERIALS AND METHODS

Observations of the distribution of WSMV, based on symptoms, were made in individual fields and plant samples were collected and sent to the NSW DPI Plant Health Diagnostic Service (PHDS) laboratory at Camden for testing for the presence of WSMV. The presence or absence of WSMV was confirmed by negative staining under an electron microscope, or by double antibody sandwich ELISA (Agdia[®], Elkhart, Indiana, USA), or both.

RESULTS

During 2008, 95 wheat plant samples were sent to the PHDS laboratory by NSW DPI district agronomists and commercial agronomists from sites across NSW. Of these samples, 73 were tested for WSMV, of which 47% were diagnosed as positive. WSMV was frequently observed in scattered plants but also occurred in small to large patches and along field edges. Overall in 2008, WSMV appeared to be widespread in wheat crops on the western slopes for the length of NSW, in central NSW up to the Liverpool Plains.

In early August 2008, a crop of wheat (cv. Sunbri) near Coonamble in northern NSW was reported to have a section at one end of the paddock with yellow plants that were also around only half the height of the rest of the crop. This area extended out about 80 metres off a fence line. Subsequently, there were further reports of individual scattered plants within paddocks with yellowing along the length of their leaves from that region, with confirmation of WSMV in the laboratory.

DISCUSSION

The scattered distribution of individually infected plants within paddocks suggests that low levels of seed transmission may have been the primary source of infection. Infected crops would potentially have included both grower-retained seed and some commercially purchased seed. The most likely scenario is that infection by WSMV occurred late (spring) in these regions in 2007, and thus went unnoticed. However, these later infections would still result in low levels of seed transmission which led to the situation observed in 2008.

It was clear that in one crop at least (the Sunbri crop near Coonamble) the presence of WCM caused considerable spread

of the virus resulting in significant yield loss. The disease distribution in that crop was consistent with WCM having migrated from dying summer grasses in the adjacent paddock some time after sowing. However, individual samples of scattered plants that had symptoms of WSMV were collected from the other side of the Sunbri paddock, where the WCM was not observed, and these plants also tested positive for the WSMV. These observations suggest that this Sunbri crop had low levels of seed-borne infection across the whole paddock, with WCM contributing to a very high incidence of the disease in the large section at one end. In other crops that had low levels of planting seed infected with WSMV with minimal spread by WCM, then yield loss would have been minimal in 2008.

In the original report of seed transmission in wheat from WA rates of 0.2–0.5% were recorded with up to 1.5% in individual genotypes (1). Later work from NSW published in 2008 found similar levels of seed transmission of 0.4% in artificially inoculated wheat cultivars with extensive streaking symptoms and a maximum of 0.22% in seed collected in 2005 from commercially infected wheat crops from southern NSW (2).

Even though these levels of seed transmission appear low, a rate of 0.4% in a wheat crop typically targeting 70–100 plants/m² would represent between 2800 to 4000 infected seedlings per hectare. Seed transmission is therefore, potentially an important factor in introducing WSMV into new locations but its importance in initiating an epidemic of WSMV remains unclear. Viral spread across a paddock from individual scattered plants arising from seed-borne infections is required to cause major yield losses. This spread can be achieved by large numbers of WCM moving from over-summering hosts, such as volunteer wheat and grass weeds, into adjacent wheat paddocks, as occurred in the Sunbri crop at Coonamble. At present it is not known whether the WCM acquires the virus from feeding on these low levels of infected wheat seedlings scattered through the paddock with, therefore, seed transmission being critical in disease spread. Alternatively the WCM may have already acquired the WSMV from its over-summering grass or volunteer wheat host when it migrates into wheat crops, with seed transmission being relatively less important.

ACKNOWLEDGEMENTS

Funding for this study was provided through NSW DPI diagnostic surveillance. The authors kindly thank the NSW DPI district agronomists and commercial agronomists for assisting with surveillance and sample submission in 2008.

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87 Effect of white rust infection, bion and phosphonate on glucosinolates in brassica crops

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INTRODUCTION

Broccoli, Rocket and Indian mustard are Brassica crops consumed throughout the world. The major class of secondary metabolites formed in these crops are glucosinolates, many of which affect human health positively or negatively. White rust, caused by *Albugo candida*, is the most common disease affecting brassicas, although there is no information on the effect of disease on levels of beneficial and harmful glucosinolates, or their impacts on human health. In this research we describe the effect of white rust, on glucosinolate levels in leaves of these brassica crops. In addition, we investigate the effects of two activators of plant defence, Bion and phosphonate, on disease and glucosinolate levels.

MATERIALS AND METHODS

Plants. Seeds (10 per pot) of Broccoli (*Brassica oleracea*) cv. 'Greenbelt' from Terranova Seed Company and Rocket (*Eruca sativa*) obtained from Yates Seed Company were sown in 10 cm diameter pots filled with Standard UC Mix + 10 g/kg Osmocote (Scott's Australia Pty. Ltd).

Plants were grown in Growth Cabinets, with relative humidity of 90% daytime and 70% night, light intensity of 710 μ moles, temperature of 15°C with daily irrigation for 1 min for broccoli and Glasshouse with 20°C temperature and daily irrigation with overhead sprinklers twice for rocket. Chemical treatments were sprayed 10 days before inoculation. Plants were inoculated 30 days after sowing.

Pathogen. *Albugo candida* obtained from DPI Victoria (Elizabeth Minchinton) from broccoli as sporangia on fresh leaves, and from rocket from a domestic garden. The pathogens were transferred onto healthy broccoli or rocket plants every 14 days.

Chemical treatments. Twenty day old plants were sprayed with Bion (10, 25 or 100 mg/L acibenzolar-S-methyl; Syngenta) or phosphonate (0.5, 1.0 or 2.0 g/L a.i. Agrifos Supa 600; Agrichem Manufacturing Industries) until runoff, requiring approximately 25 mL/pot.

Inoculation technique. Spores were scraped off leaf pustules into sterile distilled water. After vortexing and centrifuging @2000 rpm for 5 minutes the pellet was discarded. The supernatant was incubated at 16°C for 3 h to induce zoospore release and adjusted to 10⁴ zoospores/mL, and sprayed onto all leaves of 30 day old broccoli and 20 day old rocket plants. Inoculated plants were held at 16°C and 90% RH in the growth cabinet. White powdery blisters appeared on lower surfaces of inoculated leaves 7–10 days after inoculation.

HPLC Analysis. Leaves and stems were detached and frozen in liquid nitrogen then freeze dried. The sample was then ground and 0.2 g was heated in a 90°C water bath for 10 min, then 10 mL boiling water was added to the tube and heated in the water bath for another 10 min. The samples were centrifuged for 10 min @ 3000 rpm. The supernatant was collected and the pellet was resuspended in 10 mL water, vortexed and centrifuged for another 10 minutes. The supernatants were pooled and 1 mL was filtered through 0.45 μ m nylon filters into auto sampler vials.

Samples were analysed using reverse-phase HPLC (West *et al.* 2002, modified by R. Jones, DPI Victoria, *pers. comm.*). Sinigrin was used as the internal standard and other glucosinolates (progoitrin, glucoiberin, glucoraphanin, glucobrassicin and neoglucobrassicin) were identified according to their relative retention times.

RESULTS

Table 1. Disease development on rocket

Treatment	Conc.	Days for first symptom	Yellowing	
Uninoculated, untreated		11	Nil	
Inoculated + Bion	10 mg/L	10	Nil	
	25 mg/L	12	Nil	
	100 mg/L	Nil	11	
Inoculated + Phosphonate	0.5 g/L	15	Nil	
	1.0 g/L	12	Nil	
	2.0 g/L	Nil	Nil	
Inoculated + Bion + Phosphonate	25 mg/L + 1.0 g/L	11	Nil	
	Uninoculated + Bion	10 mg/L	Nil	Nil
		25 mg/L	Nil	Nil
Uninoculated + Phosphonate	100 mg/L	Nil	11	
	0.5 g/L	Nil	Nil	
	1.0 g/L	Nil	Nil	
Uninoculated + Bion + Phosphonate	2.0 g/L	Nil	Nil	
	25 mg/L + 1.0 g/L	Nil	Nil	

The results implied that the optimum concentrations to be used in further experiments were 25 mg/L Bion and 1.0 g/L phosphonate.

GLUCOSINOLATE STUDY IN BROCCOLI

Infected leaves and roots were collected at 5 day intervals before spraying, after spraying, pre and post infection. Plants were sprayed with Bion (25 mg/L), phosphonate (1.0 g/L) or combined Bion (25 mg/L) plus phosphonate (1.0 g/L). Results that indicate differences in levels of different glucosinolates at different stages of disease and chemical applications. There were no significant differences between glucosinolates in infected and uninfected leaves on inoculated plants.

DISCUSSION

Results clearly show that there is effect of the defence activators on the symptom development. Forthcoming results will describe the effect of disease as well as the defence activators on the levels of glucosinolates.

ACKNOWLEDGEMENTS

Rodney Jones, Michael Imsic, Liz Minchinton (DPI Vic).

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55 Fertilisation with N, P and K above critical values required for adequate plant growth influences plant establishment of cotton varieties in fusarium infested soil

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INTRODUCTION

Fertiliser recommendations are developed to optimise nutrient uptake and provide the crop with adequate nutrients for normal growth and yield. Once critical levels of nutrients are met, no response to yield is expected from further nutrient application, but there may be other benefits. In some instances, nutrient applications higher than those needed for optimum growth may result in improved disease resistance. The overall aim of this work is to determine the effect of N, P and K fertilisation on nutrient uptake, plant establishment, disease severity and yield on two cotton varieties grown in Fusarium infested soil. In this paper the effect of nutrient application on plant establishment of two varieties differing in Fusarium wilt resistance will be discussed.

MATERIALS AND METHODS

A field experiment was conducted from November 2008 to May 2009 near Cecil Plains, QLD, in soil naturally infested with the Fusarium wilt pathogen. Soil cores were collected and analysed for nutrient availability. Two cotton varieties differing in Fusarium wilt resistance were investigated: Sicala 45 BRF (F-rank 126) and Sicala 60 BRF (F-rank 102). The experimental design was factorial with 16 treatments in randomised blocks, 6 blocks per treatment. Triple Superphosphate was applied at 0, 20, 40 and 80 kg/ha; Urea with Entec at 0 and 150 kg/ha; and Muriate of Potash at 0 and 100 kg/ha. Calcium sulphate (200 kg/ha) was applied to every plot. Fertiliser treatments were applied by hand, broadcast to each plot. Hills were reformed following application. Seeds were sown at a depth of 10 cm. The experiment was irrigated and managed commercially.

RESULTS AND DISCUSSION

Nutrient availability. Availability of N, P and K of field soil exceeded the critical values required for adequate plant growth (Table 1). Therefore, application of N, P and K was considered above that required for adequate plant growth and optimal yields.

Table 1. Nutrient and pH analysis of field soil

Measurement	0–15 cm	15–60 cm	60–120 cm	Critical value
pH	8.01	8.28	8.45	-
N mg/kg	22	40	30	20–30
P mg/kg-Bicarb	29	9	7	6
K mg/kg	331	199	276	100–150

Varietal difference. Significantly more plants established for Sicala 45 BRF than for Sicala 60 BRF, highlighting the importance of planting varieties with higher F-rank in Fusarium infested soils.

N, P and K effects. Application of N at 150 kg/ha, significantly increased the number of plants established for both varieties (Table 2). N application has been associated with reduced Fusarium wilt disease in cotton. This may be due to an effect of form of N on the pathogen population in the soil.

Table 2. The effect of nitrogen (N) fertilisation on establishment of varieties Sicala 45 BRF (V1) and Sicala 60 BRF (V2)

N kg/ha	V1 F-rank 126	V2 F-rank 102
0	67 a	51 a
150	73 b	59 b

Data followed by different letters are significantly different from one another

Application of P at the highest rate, significantly increased seedling death of Sicala 60 BRF due to Fusarium wilt compared to plants that had 0 and 40 kg/ha of P applied (Table 3). There are reports in the literature of increasing P levels both increasing and decreasing severity of Fusarium wilt. Unfortunately little is understood about how P influences disease severity.

Table 3. The effect of P fertilisation on emergence and establishment of varieties Sicala 45 BRF and Sicala 60 BRF

P kg/ha	V1 F-rank 126	V2 F-rank 102
0	69 a	58 b
20	73 a	54 ab
40	68 a	56 b
80	69 a	51 a

Data followed by different letters are significantly different from one another

Cotton wilt is commonly found to be more destructive to the crop when grown on potassium deficient soils, and the application of high potash fertilisers has real value in the reduction of wilt, particularly when used on resistant varieties. In this trial K was abundant, however despite this, addition at 100 kg/ha significantly increased plant establishment of Sicala 45 BRF (Table 4).

Table 4. The effect of K fertilisation on establishment of varieties Sicala 45 BRF (V1) and Sicala 60 BRF (V2)

K kg/ha	V1 F-rank 126	V2 F-rank 102
0	68 a	54 a
100	72 b	56 a

Data followed by different letters are significantly different from one another

Interactive effects. When neither N nor K fertiliser was applied, the number of plants established was significantly reduced compared to other NK treatment combinations. For Sicala 45 BRF, when N was applied at 150 kg/ha and P at 20 kg/ha, plant establishment was significantly increased compared to all other NP treatments (data not shown). These results highlight the importance of a balanced nutrition which is required for the functioning of inherent pathogen defence mechanisms.

In conclusion, even when nutrient availability is adequate, plant establishment in Fusarium infested soil can be influenced by the application of N, P and K.

ACKNOWLEDGEMENTS

We thank the Cotton Research and Development Corporation for funding this research.

56 Eradication of *Elsinoe ampelina* by burning infected grapevine material

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INTRODUCTION

Burning infected plant material is widely used in the control and eradication of endemic and exotic pathogens. However, there is little scientific evidence to confirm that pathogens are eliminated during this process (1). We report experiments to assess the efficacy of burning as a means of eradicating pathogens from woody plants.

Black spot (anthracnose), caused by *Elsinoe ampelina*, is an important disease of grapevines worldwide (2). The fungus infects leaves, stems, petioles and berries. This pathogen was chosen as a model to develop an eradication strategy for the exotic disease black rot, caused by *Guignardia bidwellii*. Black rot has similar biology and epidemiology to black spot and could have a severe economic impact on the wine industry if it became endemic (3).

MATERIALS AND METHODS

An experiment was conducted in the Sunraysia district of Victoria. Vines (cvs Red Globe, Christmas Rose, Blush Seedless and Fantasy Seedless) were inoculated in spring 2007 by spraying a suspension of *E. ampelina* conidia on new shoots with 2–4 unfolded leaves. The shoots were covered with polyethylene bags overnight to provide high humidity to promote spore germination and infection.

In July 2008, vines were drastically pruned in an experiment to eradicate the disease. On treated vines, all plant material above the crown was removed and placed in a pit (5 x 3.5 x 0.5 m). In August 2008, six steel poles were placed upright at random within the pit. Steel mesh bags, containing infected vine canes (approx 30 g each) and temperature crayons (Tempilstik) in glass Petri dishes were attached to the poles at 20 and 50 cm above the pit floor. Another set of mesh bags was buried 5 cm below the soil surface on the floor of the pit. After the vine material was burnt, the mesh bags were collected and the ash was transferred to plastic tubes. Unburnt canes from untreated control material and buried samples were grated using a cheese grater. All samples were stored at 3–4°C until they were used.

A bioassay was conducted in a glasshouse at 22–28°C in December 2008 using potted grapevines (cv. Thompson Seedless). The three youngest expanded leaves on each shoot were sprayed with deionised water and dusted with the ash or grated vine material. Each treatment was applied to 3–4 shoots per vine and the inoculated shoots were covered with polyethylene bags. After 48 hours, the bags were removed, the leaves were sprayed again with deionised water and the bags were replaced and left overnight. The experiment was arranged as a completely randomised design with two replicate vines per treatment.

Twelve days after inoculation, the vines were assessed for symptoms of black spot. A Mann-Whitney U-test was used to analyse data.

RESULTS

The temperature crayons indicated that the fire reached in excess of 250°C and variable temperatures up to 60°C occurred 5 cm below the soil surface. No leaf symptoms developed on plants inoculated with ash whereas significant symptoms were observed on plants inoculated with grated material from the controls and less severe symptoms occurred on plants inoculated with buried cane material (Fig. 1).

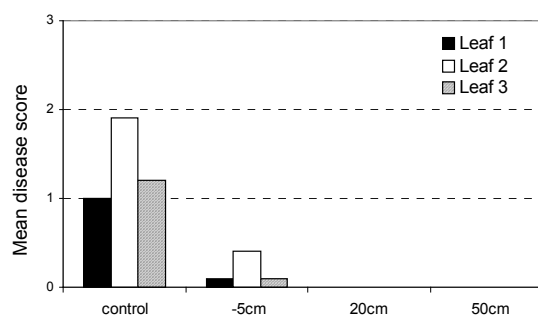


Figure 1. Mean disease score on the three newest leaves on grapevine shoots (cv. Thompson Seedless) inoculated with ash from burnt vine material positioned 20 and 50 cm above the floor of the bonfire, buried 5 cm below the soil surface under the fire or untreated (control). Samples of grapevine canes infected with *E. ampelina* in steel mesh bags were positioned at 20 and 50 cm above the ground or buried 5 cm below the surface. Data are presented for each leaf individually, with Leaf 1 being the oldest.

DISCUSSION

These results confirm the efficacy of burning infected vine material, as temperatures exceeded those that are lethal to the fungus and the bioassay verified that this was the case. However, any pathogen on debris which penetrates the soil may not be eliminated. Further research is under way to evaluate burning for eradication of the bacterium *Xanthomonas translucens* from pistachio trees.

ACKNOWLEDGEMENTS

We thank Chris Dyson (SARDI) for statistical support, members of the Department of Sustainability and Environment Victoria for assisting with the fire and the CRC for National Plant Biosecurity for funding this research.

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88 Recent plant virus incursions into Australia

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INTRODUCTION

The continuing improvement in diagnostic methods combined with the increased frequency of international travel and reduced trade barriers have all probably contributed to the upsurge in new virus records. A number of plant viruses have recently been detected in Australia for the first time. These include; *Colombian datura virus* from *Brugmansia* sp., *High plains virus* from wheat (*Triticum aestivum*), *Panicum mosaic virus* from buffalo grass (*Stenotaphrum secundatum*), *Tomato torrado virus* from tomato (*Lycopersicon esculentum*) and Tomato yellow leafcurl virus from tomato.

METHODS AND RESULTS

Colombian datura virus (CDV). A sample of *Brugmansia* sp. (angel's trumpets) with leaf mosaic symptoms was obtained from Bomaderry, NSW, in August 2007. The plant contained flexuous filamentous particles about 840 nm long and gave a positive reaction in ELISA with AGDIA potyvirus group antibodies. The C-terminal region of the coat protein and the 3' UTR were amplified by RT-PCR from an RNA extract (Qiagen RNeasy) and the products cloned and sequenced [1]. By comparison with CDV sequences on the GenBank database, the Australian sequence was 99.6–100% identical in the 3' UTR, and the amino acid sequence of the 3' portion of the coat protein was 100% identical. The consensus sequence of the Australian CDV isolate 2079 has been deposited in the GenBank database under accession FJ821796.

Panicum mosaic virus (PMV). In June, 2008, a sample of *Stenotaphrum secundatum* (buffalo grass) showing mosaic symptoms was obtained from the Sydney basin, New South Wales. The plant contained isometric virions ca. 25–30 nm diameter which were trapped by immunosorbent electron microscopy and decorated, using an antiserum to the St Augustine decline strain of *Panicum mosaic virus* (PMV). Specific PCR primers were designed to amplify the complete coat protein (CP) gene sequence of PMV. The CP of the Australian sample (PMV isolate 2349) was 90% and 85% identical to PMV GenBank Accession PMU55002 at the amino acid and nucleotide levels, respectively.

Tomato yellow leafcurl virus (TYLCV). Cherry tomato crops displaying symptoms of leaf curling, chlorosis and stunting were first reported from Pallara, Brisbane in March, 2006. Subsequent surveys indicated that the disease was common in the peri-urban areas of Brisbane, and incidences of nearly 100% were not uncommon. Infected plants gave a positive ELISA reaction with antibodies to *African cassava mosaic virus* (AGDIA), indicating the presence of a begomovirus. ELISA-positive samples were also obtained from the Lockyer Valley, Caboolture and Bundaberg areas of south-east Queensland during March to May, 2006. The TYLCV-specific PCR primers, TYLCV-F1 and TYLCV-R1 were designed to produce a 336 bp amplicon from the Rep gene. The nucleotide sequences of these amplicons from three representative isolates were ca 99% identical to those of TYLCV accessions from GenBank.

Tomato torrado virus (TToV). Since 2005, a new disease of greenhouse tomatoes has been present in the Northern Adelaide Plains, South Australia. Symptoms included chlorosis

and occasionally necrotic lesions on leaves, stunting and leaf distortion and were associated with high greenhouse whitefly populations. A low concentration of isometric virions was observed in sap preparations by electron microscopy. PCR using the TToV specific primer pairs TR1F/R and TR2F/R, designed to RNA-1 and RNA-2 respectively [2], gave amplicons of the expected size from isolate 1883 (collected in 2006) and isolate 2136 (collected in 2008). Both Australian isolates shared ca 99% nucleotide sequence identity with each other and with overseas isolates on both RNA components.

High Plains virus (HPV). HPV is a presently-unclassified, mite-transmitted virus which often occurs as mixed infections with *Wheat streak mosaic virus* (WSMV). The latter virus was recorded for the first time in Australia in 2003 [3]. Total RNA extracts of samples from the 2003 WSMV surveys were used to test for the presence of HPV. RT-PCR primers were designed to amplify part of the putative nucleocapsid gene of HPV (GenBank accession U60141). RT-PCR was done using a one-step RT-PCR kit (Qiagen). Amplicons of the expected size (483 bp) were amplified from some but not all WSMV-infected plants, indicating dual infection of some plants with HPV. The nucleotide sequences of these products were 100% identical to the published HPV sequence. HPV-infected wheat samples were obtained from experimental field plots at Roseworthy and Adelaide in South Australia and Horsham in Victoria and from a commercial crop near Moonie in Queensland.

Isolates of all the above viruses have been deposited in the Queensland Primary Industries and Fisheries Plant Virus Collection.

ACKNOWLEDGEMENTS

We thank G Ellis and E Colson for WSMV survey samples, Biosecurity Queensland staff for TYLCV survey samples and P Pezzaniti for assistance with collecting samples of TToV.

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16 Investigation of the effect of three essential oils, alone and in combination, on the *in vitro* growth of *Botrytis cinerea*

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INTRODUCTION

Many essential oils have been screened individually for their antifungal properties, but little work has been done on the combination of these oils and their potential synergistic effect on fungal plant pathogens (1). The fungus *Botrytis cinerea* Pers. ex. Fr. causes the disease grey mould worldwide on more than 100 plant species including fruit, vegetables, ornamentals and field crops, under field, glasshouse and postharvest storage conditions (2). Three essential oils, derived from clove buds, cinnamon leaves and red thyme leaves, showed the greatest inhibition of *in vitro* growth of *B. cinerea* in previous studies (2, 3). The aim of this research is to examine the effect of the essential oils clove, cinnamon and red thyme, alone and in combination, at varying concentrations, on *in vitro* growth of *Botrytis cinerea*.

MATERIALS AND METHODS

Stocks of three pure essential oils, namely clove bud (*Syzygium aromaticum* (L.) Merr. & Perry) oil, cinnamon leaf (*Cinnamomum zeylanicum* J. Presl.) oil and red thyme leaf (*Thymus vulgaris* L.) oil (Auroma Pty Ltd, Hallam, Vic.) were prepared with 0.1% v/v (final) Tween 80 used as an emulsifier. Essential oils and Tween 80 were filter sterilised into cooled molten sterilised PDA. Each oil was added, either alone or in combination in equal amounts with each other oil (two and three oil combinations), at the following concentrations: 0 (nil control), 125, 250, 500 or 1000 ppm. Mycelial plugs (6mm diameter) from the margins of actively growing, 3–4 day old *B. cinerea* cultures were inoculated centrally onto PDA plates containing the essential oils and the plates were sealed with Parafilm. Seven replicate plates were used per treatment and cultures were incubated at room temperature (~18–22°C) under natural light conditions. Mean colony diameter (mm) was measured 24 and 48 h after inoculation. The experiment was repeated. Data were analysed with an ANOVA using GenStat and means were separated using LSDs at $P=0.001$.

RESULTS

Presence of essential oils was a significant factor ($P<0.001$) in the mean colony diameter of *B. cinerea* at both 24 and 48 h. The following results are compared to the nil control at 24 h (Figure 1). PDA containing red thyme oil alone at 125 ppm and 250 ppm significantly and substantially reduced the mean colony diameter of *B. cinerea* ($P<0.001$). PDA containing cinnamon oil alone at 125 ppm significantly but only slightly reduced on the mean colony diameter of *B. cinerea* ($P<0.001$), whilst PDA containing clove oil alone at 125 ppm did not reduce the mean colony diameter of *B. cinerea*. However, PDA containing clove oil or cinnamon oil alone at 250 ppm significantly reduced the mean colony diameter of *B. cinerea* ($P<0.001$). *Botrytis cinerea* did not grow on PDA containing each oil alone at either 500 or 1000 ppm. Combining two and three oils together at 125 ppm had a significant and substantial synergistic effect on mean colony diameter ($P<0.001$), and the most effective combinations were clove and red thyme oils paired, and all three oils together. The effect of oil combinations at 250 ppm and above could not be assessed since the treatments were so effective alone. The results at 48 h and the results of the repeat experiment followed the same trends (data not shown).

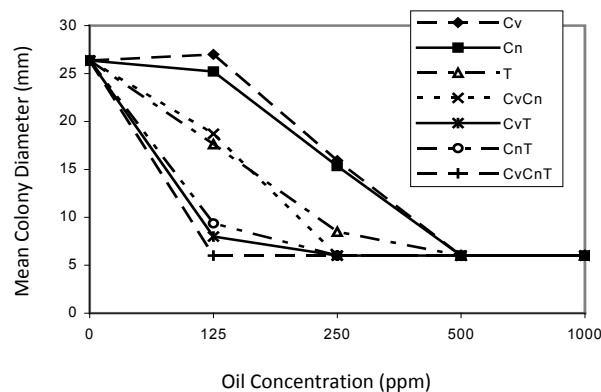


Figure 1. Mean colony diameter (mm) of *B. cinerea* grown on PDA amended with different concentrations of clove (Cv), cinnamon (Cn) or red thyme (T) oil, alone or in combination, 24 h after inoculation. LSD=0.7.

DISCUSSION

Clove, cinnamon and red thyme oils alone at various concentrations inhibited substantially the *in vitro* mycelial growth of *B. cinerea*, supporting the findings of other authors (2, 3). This is the first report of using these oils in combination to inhibit the growth of *B. cinerea*. Further work with the most effective treatment, namely clove and red thyme oils combined at 125 ppm, could include adjusting the concentrations of each oil to determine the minimum inhibitory concentration, examining if the effect is fungistatic or fungicidal and identifying the potential active constituents of each oil.

ACKNOWLEDGEMENTS

Thanks to Clare Scott and Dr Graham Hepworth (The University of Melbourne) for technical assistance and statistical input respectively.

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42 A single plant test for resistance in wheat to crown rot and root-lesion nematode (*Pratylenchus thornei*)

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INTRODUCTION

Fusarium pseudograminearum, the cause of crown rot, and root-lesion nematode (*Pratylenchus thornei*) are the most serious soil-borne pathogens of wheat in the Australian northern grain region. Only one cultivar (EGA Wylie) is both tolerant to *P. thornei* and moderately resistant to crown rot. Glasshouse methods have been developed to test wheat for resistance to crown rot (Wildermuth and McNamara 1994) and root-lesion nematode (Thompson 2008) separately. This paper reports an experiment aimed to develop a single-plant method for assessing resistance to both crown rot and *P. thornei*, which would be very valuable for accelerated wheat breeding.

MATERIALS AND METHODS

Eleven reference wheat cultivars for crown rot (susceptible Puseas and Vasco, moderately susceptible Hartog, and partially resistant Gala and 2-49), and for *P. thornei* (susceptible Gatcher, Batavia and Cunningham, and partially resistant GS50a, QT9048 and Yallaroi) were tested. Five replicate 67 mm square pots received 430 g of steam-sterilised clay-loam soil moistened to 37.5% moisture (-0.1 bar), and 10 seeds of each cultivar were placed on top. Seed was covered with 100 g dry soil (5% moisture), then 0.3 g of ground barley/wheat seed colonised with *F. pseudograminearum* was added followed by 30 g dry soil. After 7 days in a glasshouse at 25°C, top watering of the pots to 37.5% moisture was commenced. After 3 weeks, soil was washed away from the seedlings and the first three leaf sheaths were rated for crown rot symptoms on a 1 to 4 scale and summed (max score = 12).

After the crown rot test, four plants from each pot, with roots trimmed to 3 cm, were planted individually in pots of 330 g steamed vertosolic soil (Irving Series), watered and inoculated with a suspension of *P. thornei* to provide 10,000/kg soil. The plants were placed in a growth room for 4 days after which permanently wilted leaf tissue was cut off. Plants were then grown in a glasshouse with temperature at 22°C and constant 2 cm soil water tension (85% moisture). The pots received three drenches with 0.1% (w/v) benlate over 6 weeks to prevent crown rot developing further. After 16 weeks, a 150 g subsample of soil and roots from the bottom half of the pots was extracted for nematodes in Whitehead trays. *P. thornei* were counted in a 1-ml Hawksley slide under a compound microscope and expressed as number/kg soil and transformed by $\ln(x+c)$ for analysis of variance.

RESULTS

The crown rot standard cultivars performed as expected, with 2-49 and Gala relatively resistant, and Hartog, Vasco and Puseas of increasing susceptibility (Fig. 1). All *P. thornei* standard cultivars were relatively susceptible to crown rot. Results for *P. thornei* are given in Fig. 2 in log units. Backtransformed values ranged from 16,150 *P. thornei*/kg soil for QT9048 to 136,380 for Puseas. The standard cultivars for *P. thornei* performed as expected with GS50a, QT9048 and Yallaroi being relatively resistant, and Batavia, Gatcher and Cunningham being relatively susceptible to *P. thornei*. Hartog produced intermediate numbers of *P. thornei* as expected from previous experiments. All of the other crown rot standard cultivars were relatively susceptible to *P. thornei*.



Figure 1. Crown rot ratings of seedlings

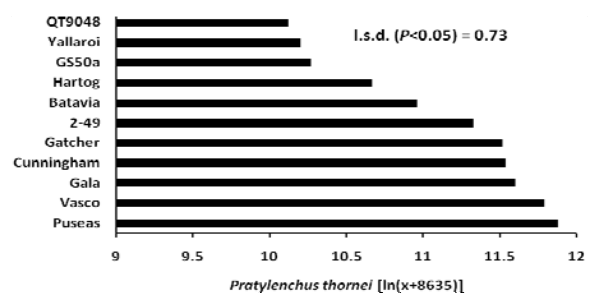


Figure 2. Number of *Pratylenchus thornei* after 16 weeks

DISCUSSION

This initial experiment shows it is possible to screen individual plants for resistance to both crown rot and *P. thornei*. The approach taken was first to test plants for crown rot by a standard method, then transplant them for a nematode resistance test. This was not ideal in that the plants suffered considerable transplanting stress and the method was labour intensive. Despite this, meaningful results were obtained for resistance to *P. thornei*. Modification of the methods should be possible to obtain an effective single plant test for resistance to both crown rot and *P. thornei* without the need to transplant.

ACKNOWLEDGEMENTS

We thank M. Brady, T. Bull, T. Clewett, S. Coverdale, M. Davis, M. Harris, N. Seymour, J. Sheedy, K. Trackson, G. Wildermuth and J. Wood for their input to this study.

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40 A single plant test for resistance to two species of root-lesion nematodes and yellow spot in wheat

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INTRODUCTION

Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and the stubble-borne fungal disease yellow spot (*Pyrenophora tritici-repentis*) cause substantial loss of wheat production in the Australian northern grain region. While some wheat varieties have partial resistance or tolerance to some of these diseases none has resistance to all. If varieties could be produced that combine resistance to all these diseases then the savings to the wheat industry would be very great.

Phenotyping for multiple diseases on a single plant could be a valuable method for rapidly breeding multiple disease resistant wheat varieties. A method has been developed to test for resistance to *P. thornei* and *P. neglectus* simultaneously (Huang *et al.* 2005) and in this study we extend it to include yellow spot.

MATERIALS AND METHODS

Forty-one wheat cultivars were subjected to three inoculation treatments (i) root-lesion nematodes (*P. thornei* and *P. neglectus*), (ii) yellow spot, and (iii) root-lesion nematodes and yellow spot together. Five replicates were grown as single plants in individual pots of 330 g of steam-sterilised vertosolic soil. Nematode inoculum of the two species was produced separately, and mixed in suspension prior to inoculating 5,000/kg soil of each species at sowing. The plants were grown in a glasshouse with soil maintained at 22°C and 2 cm water tension. At the 2-leaf stage, the seedlings of the yellow spot treatment were spray inoculated with field-collected *Pyr. tritici-repentis* conidia (0.45 mg conidia/mL). Inoculated seedlings were held in a mist chamber for 40 h, and then another 4 days in a growth room with sprinklers operating for 3 mins every 12 hrs, and temperature at 23.5°C. The plants were rated for combined chlorosis and necrosis of the leaves on a 1 (susceptible) to 9 (resistant) scale. All pots were returned to the glasshouse and laid out in a split block design. After 16 weeks from sowing, nematodes were extracted from the soil and roots by the Whitehead tray method. *Pratylenchus thornei* and *P. neglectus* were identified on morphology and counted under a compound microscope. Nematode numbers [after transformation by $\ln(x+c)$] and yellow spot ratings were analysed by ANOVA. Mean values of the 41 wheat lines were used in regression analyses.

RESULTS AND DISCUSSION

There was good discrimination between wheat lines for yellow spot ratings ($P < 0.001$) and a highly significant regression relationship ($P < 0.001$) between yellow spot ratings in the presence and absence of *Pratylenchus* inoculum (Fig. 1). This indicated that a systemic resistance was not induced and that yellow spot resistant and susceptible wheats could be reliably identified in the presence of the nematodes.

The wheat cultivars inoculated with yellow spot or not were ranked similarly for *P. thornei* resistance ($R^2 = 0.7756$, $P < 0.001$) or *P. neglectus* ($R^2 = 0.484$, $P < 0.001$) or for total *Pratylenchus* ($R^2 = 0.7738$, $P < 0.001$) (Fig. 2). Numbers of *P. thornei* and *P. neglectus* in the treatment also tested for yellow spot resistance were significantly lower than in the nematode only treatment. This effect was not correlated with the yellow spot resistance

rating of wheat lines. It was probably due to the changed growth conditions for conducting the yellow spot test resulting in less reproduction compared with the plants kept in the glasshouse.

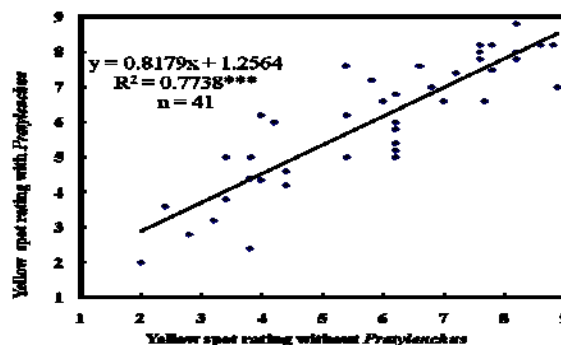


Figure 1. Highly significant regression relationship between ratings for yellow spot of 41 wheat lines when tested either without (x axis) or with *Pratylenchus* (y axis).

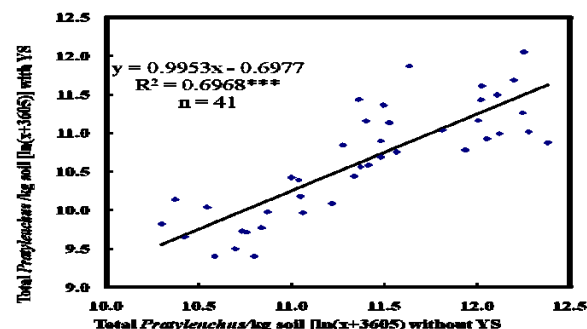


Figure 2. Highly significant regression relationship between number of *Pratylenchus* produced by 41 wheat lines when tested either without (x axis) or with yellow spot (y axis)

These results indicate that simultaneous testing of single plants for resistance to *P. thornei*, *P. neglectus* and yellow spot is feasible. With further refinement this method could improve the efficiency of breeding multiple-disease resistant wheats which would be of great value to Australia.

ACKNOWLEDGEMENTS

We thank Megan Brady for technical assistance.

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90 Role of nematodes and zoosporic fungi in poor growth of winter cereals in the northern grain region

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INTRODUCTION

The endoparasitic root-lesion nematodes *Pratylenchus thornei* and *P. neglectus* and the ectoparasitic stunt nematode *Merlinius brevidens* occur widely in the northern grain region of Australia. Grain loss in wheat has been well characterised for *P. thornei* in the northern region (Thompson *et al* 2008) and for *P. neglectus* in the southern and western regions (Vanstone *et al*, 2008). Information on the role of *Merlinius brevidens* is sparse although it has been shown to cause yield loss of wheat in the USA (Smiley *et al.* (2006), particularly when associated with the zoosporic fungus *Olpidium brassicae* (Langdon *et al.*1961). Following diagnosis of high populations of *M. brevidens* associated with poor crops of winter cereals in 2007 a glasshouse experiment was conducted in 2008 to explore further the reasons for the poor growth

MATERIALS AND METHODS

About 50 kg of soil was collected (on a grid of 36 positions within a 1,920 m² area) from each of nine fields in the northern grain region located from Garah in northern NSW to Wondai in Qld. These sites were selected on the basis that *M. brevidens* and/or *Olpidium* sp. had been detected in poorly growing cereals in the field or on the farm previously. The soil from each site was mixed, sieved and about half was partially sterilised by steam at 70°C for 45 min. Quantities of each soil (330 g OD equivalent) were mixed with 1 g of Osmocote® [Native Gardens plus micronutrients (17–1.6–8.7 NPK)] slow-release fertiliser and placed in 5 cm-square plastic pots suitable for bottom watering. Eighteen pots of each of sterilised and unsterilised soil were prepared to allow for growing 3 replicates of 3 cereals (wheat cv. Strzelecki, barley cv. Grout and oats cv. Coolibah) at 2 moisture tensions (2 cm and 7 cm) and 2 harvest times (8 and 16 wks). The pots were placed on strips of capillary matting for each soil type and on separate benches for sterilised and unsterilised soil and for the two water tensions. A single plant per pot was grown, with the glasshouse temperature kept below 25°C by evaporative coolers. At each harvest, plant tops were dried at 85°C for 4 days and weighed. Soil and roots were removed from the pots, photographed and split longitudinally. Roots were extracted from one half of the pots, blotted, weighed, and a subsample stained with trypan blue. Soil and roots from the other half were broken into pieces <1 cm and a subsample extracted for nematodes by the Whitehead tray method.

RESULTS AND DISCUSSION

All three cereals responded to soil sterilisation with mean plant dry weight in unsterilised soil ranging from 44 to 95% of that in sterilised soil when grown at 2 cm water tension (Fig. 1). The effects were relatively similar at the two water tensions of 2 and 7 cm (Fig. 1). Root systems were considerably reduced in the unsterilised soil compared with the sterilised soil.

Observations of the stained roots under the microscope showed the presence of zoosporangia and encysted zoosporangia similar to those of *Olpidium radiale* and *Olpidium brassicae* as well as *Pythium* oospores. High populations of *M. brevidens* and of *P. thornei* were present in samples extracted at 16 weeks.

The work is still in progress and this is a preliminary report.

It does, however, indicate poor root health of cereals growing in the northern region that may be due to the combined effects of nematodes and zoosporic fungi.

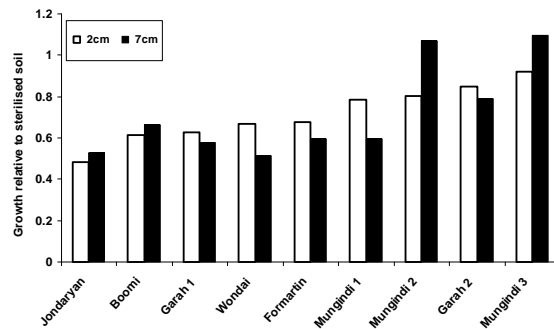


Figure 1. Response at 8 weeks in growth at two water tensions of winter cereals (mean of wheat, barley and oats) to sterilisation of soil from 9 farms in the northern grain region

ACKNOWLEDGEMENTS

We thank graingrowers for permission to sample their fields and GRDC for funding.

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41 Sources of resistance to root-lesion nematode (*Pratylenchus thornei*) in wheat from West Asia and North Africa

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INTRODUCTION

The root-lesion nematode *Pratylenchus thornei* occurs widely in the northern grain region (northern NSW and southern and central Qld) causing considerable economic loss in wheat production. Current management tools are hygiene with farm machinery to prevent transfer of infested soil, crop rotation and growing tolerant wheat varieties (Thompson *et al.* 2008). More effective control of the nematode populations could be achieved if resistant cultivars were available.

To obtain novel sources of resistance we tested two collections of wheat from the West Asia and North Africa (WANA) region. The A.E. Watkins Collection was made in Cambridge, UK, in the late 1920s and early 1930s with landrace wheats from many countries of the world (Miller *et al.* 2001). The R.A. McIntosh Collection was made in 1993 at University of Sydney with wheats from WANA countries for studies on rusts and flag smut

MATERIALS AND METHODS

Wheat Accessions. The WANA wheats tested comprised 148 bread wheat (*Triticum aestivum*) and 139 durum wheat (*Triticum turgidum* spp. *durum*) accessions from the Watkins Collection and 59 bread and 43 durum accessions from the McIntosh Collection.

Resistance Experiments. Initially each of the above collections was tested for resistance to *P. thornei* in two separate glasshouse experiments that included the reference standards GS50a (a partially resistant bread wheat) and three susceptible wheat varieties Gatcher, Suneca and Potam. A number of bread and durum wheat accessions that produced nematode numbers not significantly different from GS50a were retested for resistance in a third experiment.

Resistance test methods. The wheat accessions were grown as 3 replicates in pots of steamed vertosolic soil (1 kg soil in Experiments 1 and 2 and 650 g in Experiment 3), inoculated with *P. thornei* at a rate of 2,500/kg soil. The soil was fertilised to provide N, P, K, S, Ca and Zn and was watered to pF2 (56% moisture). The experiments were laid out in randomised blocks in an evaporatively cooled glasshouse. In Experiment 3, the soil and root temperature was kept at 22°C with pots in glasshouse waterbaths. After 16 weeks growth, one half of the soil and roots was removed, broken to < 1 cm manually and 150 g extracted for nematodes in Whitehead trays. Nematodes were counted under a microscope and expressed as *P. thornei*/kg soil (oven dry equivalent). Data were transformed by $\ln(x+1)$ for ANOVA and calculation of *F*.s.d. Backtransformed means and reproduction factors (RF = final number of *P. thornei*/ initial number) were calculated.

RESULTS AND DISCUSSION

Experiments 1 and 2. As a group, the bread wheats were significantly ($P < 0.001$) more susceptible to *P. thornei* than the durum wheats with backtransformed means for *P. thornei*/kg soil of 52,051 for bread wheats and 38,560 for durum wheats in the Watkins Collection, and 34,200 for bread wheats and 21,268 for durum wheats in the McIntosh Collection.

Experiment 3. Thirteen WANA bread wheats (Fig. 1) and 10 durum wheats (data not shown) had *P. thornei* numbers that did not differ from GS50a in two experiments. All Australian bread wheats were susceptible whereas the two Australian durums were as resistant as GS50a.

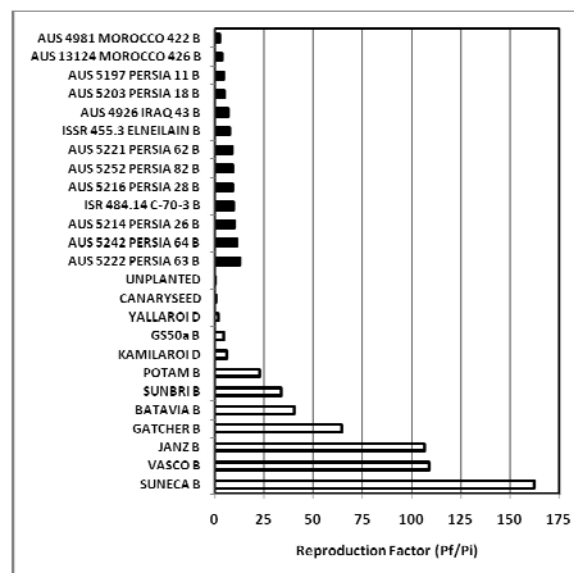


Figure 1. Reproduction factor of WANA bread wheats (black bars) that did not differ significantly from GS50a in comparison with reference standard wheats (open bars) from the northern grain region. The letters B and D after names indicate bread and durum wheats respectively.

The identification of additional sources of resistance in bread wheat to *P. thornei* provides greater options for producing Australian wheat varieties with greater levels of resistance to *P. thornei* than in current varieties.

ACKNOWLEDGEMENTS

We thank Michael Mackay and Greg Grimes of Australian Winter Cereals Collection, and Professor Bob McIntosh University of Sydney or provision of seed

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89 Sugarcane downy mildew: development of molecular diagnostics

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INTRODUCTION

Sugarcane downy mildew (SDM) is currently one of the most serious diseases at the Ramu sugarcane plantation in Papua New Guinea. SDM is rated as a high priority pathogen for the Australian sugar industry.

SDM can be caused by several fungal species in the genus *Peronosclerospora* including *P. sacchari*, *P. spontanea* and *P. philippinensis* (1). *P. sacchari* was eradicated from Australia during the 1950s by resistant varieties and roguing of remaining infected plants (2). It is estimated that 60% of current commercial sugarcane varieties in Australia are intermediate or susceptible to SDM.

The species of *Peronosclerospora* are difficult to distinguish by traditional taxonomy, so molecular diagnostics would be useful to conclusively identify the species during a disease incursion.

Peronosclerospora diagnostics using both hybridisation (3) and PCR (4) have been developed in the USA; however the number of isolates available was limited in these studies (D. Luster, pers. comm.). Therefore molecular diagnostics need to be verified on a larger sample size of *Peronosclerospora*, including Australian domestic and exotic species.

MATERIALS AND METHODS

Target genes and isolates. DNA sequences from isolates held at USDA-ARS (Ft Detrick, MD) were used to develop primers for regions that showed variation (Table 1). Species used to design and test the primers are shown in Table 2.

Table 1. *Peronosclerospora* genes used as primer targets

Gene	Predicted outcome
ITS1*	General primers; should amplify all
Actin*	Designed to amplify all Oomycetes
EF1*	Variation within and between species; may not be good for diagnostics
Beta-tubulin	High variation; unsure as to efficacy of primers.
COX-1	Size differential between species, may not amplify some species

* designed by Dr Clint Magill, Texas A&M University
 (ITS1: ribosomal DNA first internal transcribed region; EF1: Transcription Elongation Factor 1 alpha; COX-1: Cytochrome oxidase-1)

Table 2. *Peronosclerospora* species used in this study

Species	Detail
<i>P. sacchari</i>	Sequence from up to 3 isolates
<i>P. philippinensis</i>	Sequence from up to 3 isolates
<i>P. sorghi</i>	Sequence from up to 3 isolates
<i>P. maydis</i>	Sequence from up to 6 isolates
<i>P. sacchari</i>	2 herbarium isolates
<i>P. sorghi</i>	1 herbarium isolate.
<i>P. eriachloae</i>	1 herbarium isolate
<i>P. noblei</i>	1 herbarium isolate
<i>P. sp.</i>	2 unknown herbarium isolates

Amplification. Theoretical amplification was done using *Peronosclerospora* gene sequences from isolates held at USDA-

ARS. *Peronosclerospora* isolates from the QDPI&F Plant Pathology Herbarium were used to test efficacy of primers.

Analysis. DNA was extracted from isolates using QIAGEN DNeasy kit. PCR was done using primers developed for the genes of interest, and the results analysed on agarose gels.

RESULTS AND DISCUSSION

Amplification with ITS-1 was used as a positive control to ensure that amplifiable DNA was obtained after extraction. Unfortunately, one of the *P. sacchari* herbarium isolates was not amplifiable. Further investigation is needed to test this isolate.

The general Oomycete primers (actin) gave amplified products in some, but not all species. This is unexpected, because this primer set was designed to amplify products from all Oomycetes.

The primers for EF1 were tested and a high degree of variation observed, with complex banding patterns between all species tested. This primer set is likely to not be useful for diagnostics unless further sequencing reveals diagnostic differences.

Beta-tubulin primers showed differential amplification between species, however some unexpected complex banding was observed. Further investigation of this primer set is also required.

The Cox-1 primer set was designed to show differences between species, and it showed promise as a diagnostic, however the amplification of *P. sacchari* remains inconsistent.

Future work is planned to obtain more isolates of the causal agents of SDM to troubleshoot and refine the molecular diagnostic test.

ACKNOWLEDGEMENTS

Thanks to: Dr Clint Magill, Dr Ram Perumal (Texas A&M University) and Dr Doug Luster (USDA-ARS, Ft Detrick) for initial sequencing information; to Dr Roger Shivas (QDPI&F Plant Pathology Herbarium) for isolates and collaboration. This project was partially funded by an SRDC Travel and Learning Opportunity scholarship.

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43 Effect of irrigation method on disease development in a carrot seed crop

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INTRODUCTION

For carrot (*Daucus carotae* L.) seed production in Canterbury, New Zealand, overhead irrigation is commonly used from mid-November until March to prevent soil moisture deficits. However this irrigation method may assist the spread of plant pathogens such as *Alternaria radicina*/*A. daucii*/*Cercospora carotae* within the crop. We compared the effects of overhead irrigation and drip (T-Tape) irrigation on disease level in a carrot seed crop in Canterbury.

MATERIALS AND METHODS

A field assessment of the effect of irrigation method (overhead vs. drip) was made in the 2007/08 season. An 8 ha field was divided into two; 4 ha was irrigated via a conventional overhead system, and 4 ha was irrigated via a drip (T-Tape) system. The drip lines were dug 3–4 cm below the soil surface in early November. Soil moisture was monitored using a neutron probe and irrigation applied as required. Irrigation treatments were not replicated, but within each 4 ha block, twelve 30 m² plots were selected at random and used for three assessment of foliage disease symptoms using a 1 to 10 rating scale where 1= no symptoms and 10= dead plant and an assessment of root disease symptoms using a 0 to 4 rating scale where 0= no evidence of root infection and 4= shoulder region of root completely girdled with black rot. At maturity ten primary umbels were hand harvested from each plot and hand threshed. Seeds were then dried at 30°C to bring the seed moisture level down to 8%, hand rubbed to remove spines and thoroughly mixed. Hands were sterilised with 90% ethanol to prevent contamination while handling different seed samples. For seed infection, 100 carrot seeds per plot were plated onto a semi selective agar media for *A. radicina*, and incubated at 27°C in 24 h dark for 14 days. For seed germination, 100 seeds per plot were placed between moist germination paper towels and incubated at 20°C in 24 h dark for 14 days before evaluation. The collected data were statistically analysed using an unpaired t-test.

RESULTS

The carrot plants from drip irrigated blocks had significantly less foliage infected by *A. radicina*/*A. daucii*/*Cercospora carotae* at all three assessment times (Fig 1, $P < 0.05$) and slightly less black rot on roots (Fig 1, $P < 0.05$) than plants from the overhead irrigation block. Seeds harvested from the drip irrigated block had a lower incidence of *A. radicina* (9.5% cf 14.5%, Fig 2, $P < 0.05$) and a slightly higher germination (75% cf 66%, Fig 2, $P < 0.05$), mostly because there were fewer dead seeds (9% cf 14.5%, Fig 2, $P < 0.05$) than the seeds from the overhead irrigated block.

DISCUSSION

These preliminary results indicate that drip (T-Tape) may have an advantage over overhead irrigation if pathogens are present. Drip irrigation did not wet carrot foliage, and thus provided an environment less favourable for pathogen spread within the crop. These results support previous research findings in Central Oregon (USA) on effects of irrigation method in the carrot seed crop, where it was shown that drip irrigation reduced disease level, increased seed yield and improved water use efficiency (1, 2, 3). As the present work did not include replication of the irrigation treatments, further work will be required to determine

whether irrigation method does have a significant impact on disease levels and seed yield and quality in Canterbury carrot seed crops.

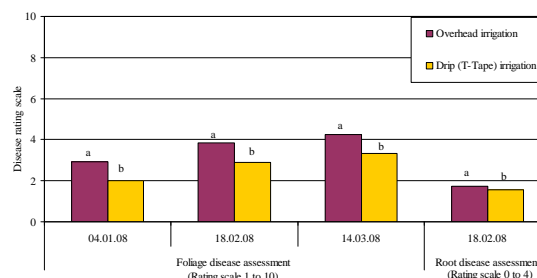


Figure 1. Effect of irrigation method on disease recorded on carrot plants.

A statistical analysis of foliage and root assessment at different times was done separately.

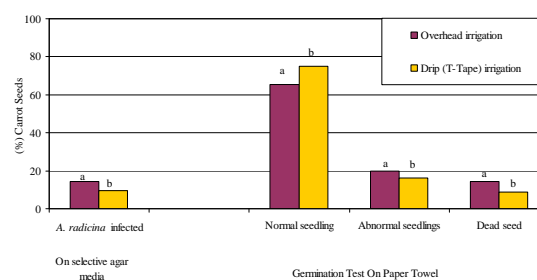


Figure 2. Effect of irrigation method on seed borne *A. radicina* and seed germination.

A statistical analysis of each parameter was done separately.

For each assessment, a different letter above the bar indicates a significant difference according to the unpaired t-test ($P \leq 0.05$).

ACKNOWLEDGEMENTS

We thank the Foundation for Research Science and Technology and Midlands Seed Ltd who provided funding for this research. Thanks also to Netafim NZ Ltd, KB Irrigation and HydroServices Ltd staff for their assistance with irrigation products and irrigation scheduling.

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91 Pathogenicity of *Radopholus similis* on ginger in Fiji

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INTRODUCTION

Radopholus similis was first associated with a disease of ginger (*Zingiber officinale*) in the early 1970s, when stunted, chlorotic, low yielding crops in Fiji were found to be infested with the nematode (1). Nematodes were observed in small, shallow, water soaked lesions on the rhizome surface, and these lesions eventually enlarged until the rhizome was destroyed.

Although *R. similis* was considered primarily responsible for the symptoms observed, secondary organisms were also thought to be involved (1). This study sought to confirm this by examining the pathogenicity of the nematode on ginger in a more controlled environment.

MATERIALS AND METHODS

Twenty 4 L pots were filled with autoclaved potting mix and planted with a *Radopholus*-free 'seed piece' of ginger (a section of rhizome used as planting material). Pots were then transferred to a glasshouse and 6 weeks later, half the pots were inoculated with 1,500 *R. similis*. The nematode was obtained from a ginger farm at Veikoba, Fiji and had been multiplied in the laboratory on sterile carrot tissue. Fifteen and 20 weeks after pots were inoculated, the number of yellowing or dead shoots in each pot was recorded, above-ground biomass in five inoculated and five control pots was measured and symptoms on seed pieces and newly-developing rhizomes were assessed. Nematodes were extracted by spreading macerated rhizomes or 200 mL samples of soil and roots on an extraction tray and recovering them on a 38µm sieve.

Portions of seed pieces and rhizomes showing symptoms possibly caused by *R. similis* were assessed by removing small pieces of tissue from affected areas, macerating them in water and checking for nematodes after 24 hours. Discoloured tissue was also checked for fungal pathogens known to cause discoloration or rotting of ginger (i.e. *Fusarium* and *Pythium*) by placing small pieces of tissue onto potato dextrose agar (PDA) or corn meal agar with carbendazim, ampicillin, rifampicin, pentachloronitrobenzene and pimaricin (CARPP), and observing plates after 24 and 48 hours.

RESULTS

Non-inoculated plants grew normally and after 15 weeks they had several healthy green shoots up to 90 cm long. In contrast, the stem bases and lower leaves of 6 of the 10 inoculated plants were yellow and in some cases the affected shoots had died. Yellowing was first observed about 12 weeks after inoculation and at 15 weeks, 40% of shoots were chlorotic or dead. By 20 weeks, three inoculated plants had died, shoots on the remaining plants were dying back, rhizomes and seed pieces were discoloured or badly rotted and plant biomass was significantly reduced relative to the control (Table 1).

Observations on tissue collected from affected plants showed that *R. similis* was present in the lowest leaf sheaths, in the collar at the base of the shoot, and in rhizome tissue at the point where shoots emerged from the rhizome. The nematode was also recovered from sunken lesions and blackened tissue on the rhizome surface, from discoloured tissue that extended 1–3 mm

into the rhizome, and from seed pieces. In some cases, the nematode population in parts of a seed piece or rhizome was as high as 500 *R. similis*/g. *Fusarium*, *Pythium* or other fungi were not isolated from discoloured tissues. Estimates of the number of *R. similis* recovered after 20 weeks indicated that significant nematode multiplication had occurred in roots, seed pieces and rhizomes (Table 2).

Table 1. Effect of *Radopholus similis* on ginger 20 weeks after plants growing in potting mix were either inoculated with the nematode or left uninoculated

Treatment	Dry wt. shoots (g)	Fresh wt. seed piece (g)	Fresh wt. rhizome (g)
Control	79.2 a	52.8 a	54.4 a
<i>R. similis</i>	7.5 b	23.7 b	24.5 b

Numbers in the same column followed by different letters are significantly different (P= 0.05)

Table 2. Numbers of *Radopholus similis* recovered 20 weeks after ginger plants were inoculated with 1,500 nematodes or left uninoculated

	No. <i>R. similis</i> females		
	/seed piece	/rhizome	/pot (soil + roots)
Control	0	0	0
<i>R. similis</i>	397	884	15,628

DISCUSSION

Our results suggest that *R. similis* multiplies initially on seed pieces and roots and then invades newly-developing rhizomes. The nematode first seems to feed on outer parts of the rhizome and the resulting damage leads to yellowing at the base of shoots and of the lower leaf sheath. As more tissues are destroyed, older leaves turn yellow, shoots eventually collapse and discoloration extends further into the rhizome. The end result is that plants eventually die and the rhizome is totally destroyed.

Given the results of our experiment, we suggest that *R. similis* is a pathogen of ginger in its own right. The nematode is capable of killing plants and destroying rhizomes, with secondary organisms playing little role in symptom development.

ACKNOWLEDGEMENTS

Funding from ACIAR is gratefully acknowledged.

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17 Survival of the pistachio dieback bacterium in buried wood

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INTRODUCTION

Pistachio dieback is a bacterial disease causing internal staining, trunk and limb lesions, decline, dieback and, in some instances, death of pistachio trees (1). Apparently endemic in Australia, the causal agent is a strain of *Xanthomonas translucens*. It is a vascular pathogen that provides a local model to assess the effectiveness of existing eradication strategies for systemic bacterial pathogens of woody perennials which are considered high-priority emergency plant pests in Australia. Burial and burning are two accepted means of disposal of diseased plant material. However, there is little or no information on the survival of bacterial pathogens following burial or burning of infected wood. The efficacy of burial and burning as means of safe disposal of diseased wood is being evaluated. This paper reports the survival of the pistachio dieback bacterium in buried wood to date.

MATERIALS AND METHODS

Infected pistachio wood was collected from the Waite Campus orchard in August 2008. Staining was assessed and the presence of *X. translucens* was confirmed by culturing on antibiotic benlate sucrose peptone agar (ABSPA, 1) and by polymerase chain reaction (PCR) using strain-specific primers (2).

Methods adapted from those described by Naseri *et al.* (3) were used. Plastic mesh bags containing pieces of infected wood or mulched pistachio wood (approx 20 g each) were buried 10 cm deep in pots filled with orchard soil or left on the soil surface. Pots were placed outdoors in August 2008 and retrieved monthly to assess survival of *X. translucens*. Upon retrieval, each piece of wood was cut into half. One half was surface-sterilised and bacterial suspensions obtained by soaking in sterile distilled water overnight at room temperature. Suspensions were streaked on several culture media amended with various antibiotics and incubated at 28°C for 2–8 days. Suspensions prepared for culturing were also assayed by PCR using strain- and species-specific primers.

The remaining wood samples were sliced into pieces (1–1.5 mm thick), surface sterilised and placed directly onto V8 juice agar amended with streptomycin for fungal isolation and nutrient agar (NA, Oxoid) amended with benlate for bacterial isolation. Plates were incubated at 25°C for 3–5 days, then bacteria and fungi were subcultured onto NA and PDA, respectively. To screen for antagonism, a suspension of *X. translucens* was spread on sucrose peptone agar plates an hour before applying to the centre of each plate a small amount of bacteria isolated from the wood. Plates were incubated at 28°C and observed for 3–7 days.

RESULTS

X. translucens was detected by PCR with species-specific primers in most wood samples up to 7 months after burying in soil or placing on the soil surface. *X. translucens* was rarely detected by culturing, as plates were often over-

grown by soil and/or wood microorganisms. However, *X. translucens* was isolated on NA amended with ampicillin, cephalixin and gentamycin from some wood and mulch samples after 8 months buried in soil. Nutrient agar with antibiotics was

selected as the optimal culture medium for subsequent isolation of *X. translucens* from buried wood.

A number of bacteria isolated from wood, buried or left on the soil surface, produced clear inhibition zones and some prevented the growth of *X. translucens*. Fungi isolated from wood samples have been stored for future use.

DISCUSSION

Viable *X. translucens* has been detected in pistachio wood buried for 7 months. The burial experiment will continue, to examine if *X. translucens* can survive in buried infected wood for up to 2 years. In comparison, *X. campestris* pv. *campestris* survived for 507 days in cabbage stem residues buried in soil (4).

Inhibition of the growth of *X. translucens* on agar by bacteria isolated from the wood may explain the difficulty in isolating *X. translucens* from wood. Antibiotic activity of these bacteria is being assessed. Another explanation for the difficulty in isolating *X. translucens* from the wood might be that *X. translucens* enters into the viable but non-culturable (VBNC) state in response to the environmental conditions during burial. This possibility is being examined.

Frequently isolated fungi will be assessed for their ability to decompose pistachio wood.

A preliminary trial conducted in August 2008 to assess burning for disposal of diseased wood yielded no viable *X. translucens* from ash or wood buried 5 cm below the pit surface. However, eradication from debris which penetrates the floor of the pit may depend on that wood reaching the lethal temperature for the bacterium. Further experiments are planned for winter 2009 to study the effect of heat and burning on survival of *X. translucens*.

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92 The effect of dryland salinity on the diversity of arbuscular mycorrhizal fungi

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INTRODUCTION

Dryland salinity is a significant environmental problem in Australia. The large-scale removal of native vegetation and the sowing of shallow-rooted pastures and crops is a major contributor to dryland salinity. Much of the research that aims to improve land productivity and maintain biodiversity focuses on above ground biomass. Less research has investigated the deleterious effects of dryland salinity on soil microorganisms such as arbuscular mycorrhizal fungi, considered essential for the establishment and growth of plants. The aim of study was to investigate the diversity of arbuscular mycorrhizal (AM) fungi from a saline agricultural field site using denaturing gradient gel electrophoresis (DGGE).

MATERIALS AND METHODS

Field site and sampling Soil sampling commenced after an electromagnetic survey (EM) of the saline field site and electrical conductivity (EC) testing of the soil was complete. The soil salinity ranged from non-saline (<200 mS/m) to moderately saline (500–800 mS/m) to very saline (800–1600 mS/m) and extremely saline (>1600 mS/m) based on plant salinity tolerance guidelines used in Western Australia (1). The paddock was divided into 24 plots from which soil cores (5 mm x 40 mm) were randomly taken. Soil was sampled in the summer and spring of 2007.

Trap cultures Soil removed from each of the 24 plots at both sampling times was used to set-up trap cultures using several salt-tolerant pasture and grass species. The purpose of the trap cultures is to provide conditions for maximum sporulation with the aim to increase diversity compared to that seen in field soil. Cultures were grown for 5 months before being assessed for sporulation. Sporulation was so poor for both sets of cultures that pots were re-sown and grown for an additional 4 months.

Spore extraction, DNA extraction and PCR-DGGE Spores were extracted from 50 g soil sub-samples (summer and spring field soil and trap culture soil) using wet sieving and sucrose centrifugation and DNA was extracted using the Powersoil™ DNA isolation kit (Mo Bio Laboratories Inc, USA). A nested PCR was used to amplify the partial small subunit gene 18S ribosomal DNA gene (550 bp). The first reaction employed the universal fungal primers GeoA2/Geo11 (2) and the second reaction used the fungal primer AM1 (3) and the universal eukaryotic primer NS31 (4) with an attached 5' GC clamp on the NS31 primer (NS31-GC) (5) for analysis with DGGE. Gels contained 7% (w/v) polyacrylamide (37:1 acrylamide/bis-acrylamide) and the linear gradient was 30–50% denaturant. Gels were run at a constant temperature of 60 °C for 16 hrs at 70V using Bio-rad's Dcode™ Universal Mutation Detection System (Biorad, Australia).

RESULTS AND DISCUSSION

Preliminary results suggest that genetic diversity exists across the field site irrespective of salinity levels (compare N1-7). It is hypothesised that salinity will reduce the genetic diversity of AM fungi at this field site, given that large salt scalds on the property have reduced vegetation cover. Further analysis is under way, which aims to reveal the diversity between soil samples across the salinity gradient on both a spatial and temporal scale. Cloning and sequencing will be used to identify the AM fungi

present and to provide a general picture of the diversity of AM fungi at a dryland salinity-affected site.

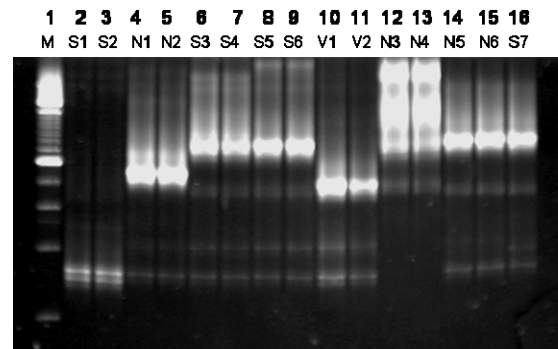


Figure 2. DGGE gel of summer field samples. Lane 1 (M) = ladder, S = slightly saline, N = non-saline, V = very saline. One sample is represented by two lanes except for lane 1 (ladder) and lane 16.

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18 Discovery of a *Ceratocystis* sp. associated with wilt disease of two native leguminous tree hosts in Oman and Pakistan

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INTRODUCTION

Ceratocystis fimbriata Ellis and Halst *sensu lato* represents a well recognised group of cryptic species that cause serious canker stain and vascular wilt diseases on a wide range of mostly woody hosts. An epidemic wilt disease, devastating thousands of mango trees, has occurred in Oman and Pakistan since 1998 (1, 2). The pathogen causing the disease was identified as the new and cryptic species *Ceratocystis manginecans* M. van Wyk, A. Al Adawi and M.J. Wingf., based on morphology and DNA sequence comparisons (2). Recently, native Ghaf (*Prosopis cineraria* L. Druce) trees in Oman began to show symptoms of wilt similar to those displayed by mangos. Intriguingly, a similar wilt diseases has been observed on native Shisham (*Dalbergia sissoo*, Roxb.) trees in Pakistan. The objective of this study was to identify the pathogen causing the wilt disease of *P. cineraria* and *D. sissoo* in Oman and Pakistan.

MATERIALS AND METHODS

During 2004–2006, samples from *P. cineraria* trees showing recent wilting symptoms were collected from Wilayat Sohar in the northern region of the Sultanate of Oman. In May 2006, samples were collected from *D. sissoo* plantations in Fasilabad, Shorkot, Chenab negar and Multan, in Pakistan. Wood samples exhibiting vascular discolouration were placed in moist chambers and incubated at 25°C for 7 days. Wood samples were also placed between carrot slices (3) to bait for the possible presence of *Ceratocystis* spp.

Morphological observations of the isolated fungi were made from cultures on 2% Malt Extract Agar (MEA) that had been incubated for 10 days at 25 °C. The ITS regions of two isolates from each of the two host species were amplified and sequenced. Sequence data were compared to those in the Genbank database using a blast search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Two isolates (CMW17225 and CMW17570) of the *Ceratocystis* sp. isolated from *P. cineraria* in Oman were tested for pathogenicity. Five seedlings, approx 2-yrs-old, were inoculated with each isolate, 15 cm above soil level. Five control seedlings were wounded and inoculated with sterile MEA. Lesion lengths were measured after 42 days.

RESULTS

Symptoms on diseased *P. cineraria* and *D. sissoo* included streaked discolouration of the vascular tissue and rapid wilting of the foliage. Cultures produced on MEA resembled those of *C. fimbriata* s. l. based on the morphology and size of the perithecia, ascospores, conidia and conidiophores. Blast searches of the ITS sequences of two isolates from *P. cineraria* showed 100% similarity with *C. manginecans*, while the two isolates *D. sissoo* shared 98% similarity with that species.

All the inoculated *P. cineraria* seedlings had vascular discolouration and 40% of the inoculated seedlings wilted and

died after four weeks. Lesions produced by isolates CMW17225 and CMW17570 were 105 and 131 mm long respectively, and significantly longer than those of the control treatments (5 mm).

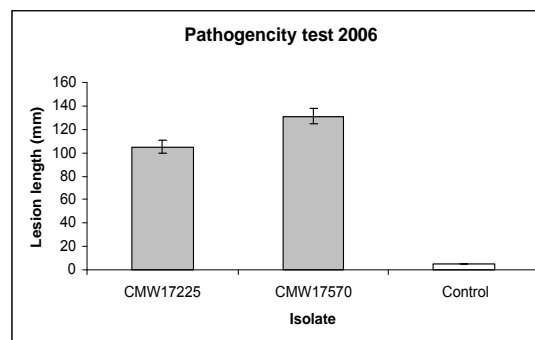


Figure 1. Mean lesion lengths on *P. cineraria* seedlings 42 days after of inoculation with two *Ceratocystis* isolates.

DISCUSSION

The results of this study have shown clearly that a *Ceratocystis* sp. is closely associated with the wilt disease of *P. cineraria* in Oman and *D. sissoo* in Pakistan. Based on ITS sequence data, this fungus is very similar, if not identical, to *C. manginecans*. Furthermore, pathogenicity tests have provided good evidence that the *Ceratocystis* sp. is the cause of the wilt disease of *P. cineraria* and most probably also *D. sissoo* trees in Oman and Pakistan.

The relatedness of the pathogen of *P. cineraria* and *D. sissoo* to *C. manginecans*, which causes a devastating disease of Mango in Oman and Pakistan, is intriguing. It has been suggested previously that *C. manginecans* is most likely an introduced pathogen in Oman and Pakistan (2). It is thus possible that this pathogen has subsequently adapted the ability to infect and kill native trees in those countries. This question deserves further and urgent study.

ACKNOWLEDGEMENTS

We thank members of the Tree Protection Co-operative Programme (TCP), University of Pretoria, South Africa, and the Ministry of Agriculture, Oman, for funding. We also thank the Nuclear Institute for Agriculture and Biology (NIAB) for facilitating surveys and shisham sample collection in Pakistan.

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93 Evaluation of plant extracts for control of sclerotinia pathogens of vegetable crops

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INTRODUCTION

Sclerotinia, caused by *Sclerotinia sclerotiorum* and *S. minor*, is one of the most damaging soilborne diseases of vegetable crops in Australia. These pathogens are difficult to control because they have a wide host range, survive in soil for many years as melanised sclerotia, and there are limited fungicide and genetic resistance options available. Beneficial management practices to reduce disease risk and IPM compatible control measures, suitable for on-farm use, are both required to achieve sustainable control of Sclerotinia. Our research is therefore evaluating a range of new practices and control methods to improve the management of Sclerotinia in vegetable production. One potential control method is the use of plant extracts with antifungal volatile compounds. For instance, isothiocyanates (ITCs) released from cruciferous plant residues during hydrolysis of glucosinolates reduced the viability of *S. sclerotiorum* sclerotia (1) and unknown volatile compounds released from fennel oil inhibited mycelial growth of *S. sclerotiorum* (2). We report here on the efficacy of two mixtures of plant extracts and one essential oil on the viability of mycelium and sclerotia of *S. sclerotiorum* and *S. minor*.

METHODS

Treatments. Voom® (mustard and other essential oils, 15–20% allyl-ITCs, Akhil), Dazitol® (mustard oil and capsaicinoids, unknown % ITCs, Champon) and bitter fennel oil (*Foeniculum vulgare*, Essential Oils of Tasmania) were tested at concentrations from 1–8% v/v. Fluke (20% allyl-ITCs) was used for comparison as standard control in soil bioassay.

In vitro tests. Mycelial plugs and sclerotia of *S. sclerotiorum* and *S. minor* isolates from bean and lettuce, respectively, were plated onto PDA amended with different concentrations of the treatments (contact diffusion). Inocula were also exposed to treatments using an inverted Petri dish assay (vapour phase). Plates were incubated for 7 days at room temperature and colony diameters recorded until growth reached the edge of the plates. Inocula that did not grow were transferred to unamended PDA to test whether the activity was fungicidal or merely fungistatic.

Soil bioassay (sclerotia). Sclerotia in mesh bags (5/bag) were exposed to treatments (vapour phase) in non-sterile sandy soil (1 kg sealed pots) outdoors for 24 hrs (n = 3). After this period, sclerotia were surface sterilised and plated onto PDA to determine viability.

PRELIMINARY RESULTS

Effect on mycelial growth in vitro. All concentrations of Voom (1%, 3%, 5% v/v) were biocidal to mycelium of both *Sclerotinia* spp, irrespective of application method. Dazitol (4, 6 and 8% v/v) was biocidal to mycelium of both spp. when using the vapour phase method. Using the diffusion method, 6 and 8% were biocidal but 4% was only fungistatic. Fennel oil (2, 4 and 6% v/v) significantly suppressed mycelial growth of both pathogens.

Effect on sclerotia viability in vitro. Voom (3% and 5% v/v) was biocidal to sclerotia of both pathogens (Table 1). At 1%, Voom was more effective in reducing the viability of sclerotia of *S. sclerotiorum* than *S. minor*. Dazitol (6% and 8% v/v) was biocidal to *S. minor* sclerotia and significantly reduced the viability of *S.*

sclerotiorum sclerotia by 78–89%. Dazitol (4% v/v) also significantly reduced sclerotia viability by 78%. Fennel oil (2, 4 and 6% v/v) had no effect on sclerotia viability.

Table 1. Mean percentage reduction of sclerotia viability *in vitro*.

Treatment (v/v)	<i>S. minor</i>	<i>S. sclerotiorum</i>
untreated	0 a	0 a
Voom 1%	11 b	100 c
Voom 3%	100 d	100 c
Voom 5%	100 d	100 c
Dazitol 4%	78 c	78 b
Dazitol 6%	100 d	78 b
Dazitol 8%	100 d	89 b

Means with the same letters are not significantly different (P<0.05).

Effect on sclerotia viability in soil. Voom (3% and 5% v/v) was more effective in reducing the viability of *S. minor* sclerotia (100% mortality) than those of *S. sclerotiorum* (78% mortality). Fluke was biocidal to sclerotia of both isolates. Fennel oil and Dazitol tested at *in vitro* concentrations were not effective in reducing sclerotia viability in soil. However, fennel oil tested at higher concentrations (>6%) significantly reduced the viability of sclerotia of both spp.

DISCUSSION

The three products tested all released volatile compounds with antifungal activity against the two *Sclerotinia* species. Voom and Dazitol, tested at concentrations considered economic for disease control, were biocidal to inocula *in vitro*. In soil, however, Voom® (15–20% allyl-ITCs) was more effective than Dazitol (unknown levels of ITCs) in reducing sclerotial viability of both pathogens. The standard treatment, Fluke, with similar levels of ITCs (20%) to that of Voom, caused total sclerotia mortality. Future studies will determine if differences in efficacies in soil were due to higher levels of ITCs released from Voom during the hydrolysis of glucosinolates. Further investigations to determine concentrations of ITCs required to achieve high levels of sclerotia mortality in soil are necessary to optimise biofumigant treatments such as plant extracts and biofumigant crops. Volatile compounds released from the low concentrations of fennel oil tested were only inhibitory to mycelial growth. Further studies of essential oils would be necessary to determine whether they have useful anti-fungal compounds at concentrations that might be biocidal to *Sclerotinia* inoculum, and if so, to determine their mode of action.

ACKNOWLEDGEMENTS

We thank Horticulture Australia Ltd and the Department of Primary Industries Victoria for financial support.

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94 First report of *Macrophomina phaseolina* on rapeseed stem in some provinces of Iran

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INTRODUCTION

Rapeseed (*Brassica napus*) is one of the most important oleaginous crops in different areas of Iran. Recently, in some cases, gray-black lesions with few microsclerotia were observed on rapeseed stem.

MATERIALS AND METHODS

In August 2006, rapeseed basal stem and taproot samples were collected from Azarbayegan sharghi (As), Ardabil (Ar), Kermanshah (Ke), Khuzestan (Ku), Fars (F) and Hamadan (H) provinces in Iran. Small pieces (5 mm) of these tissues were surface sterilised with NaOCL(1%) for 1 min and then positioned in the center of plates containing potato dextrose agar (PDA). Plates were maintained at 25°C for 4 days in the dark condition. Average diameter of 200 microsclerotia was calculated for each isolate.

RESULTS

Grey-black color mycelia with spherical and black microsclerotia observed after 5 days. Yielding fungal colonies identified as *Macrophomina phaseolina* (Tassi) Goidanich based on mycelia and size of the microsclerotia. Size of 6 isolates microsclerotia was estimated for different areas (five provinces), from 50 to 180 µm in diameter. To our knowledge, this is the first report of *M. phaseolina* on rapeseed stem (cultivars: Okapi, Zarfam, Licord and Hyola401) in mentioned provinces of Iran.

DISCUSSION

Charcoal rot on canola has been reported from Argentina (2) with the presence of microsclerotia 71–94 µm in diameter and the United States (1). Average diameter of microsclerotia in Iran was consisted: Ku-102(70–150) µm, As1- 96.7(60–150) µm, F-95(60–150) µm, Ke-92.81(50–180) µm, Ar-91.87(60–150) µm and As2- 87.03(60–180) µm. The highest and lowest size of sclerotia was related to warmest and coldest areas respectively. It seems to be diversity between Iranian isolates.

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44 First report of rapeseed blackleg caused by pathogenicity group T (PGT) of *Leptosphaeria maculans* in Mazandaran province of Iran

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INTRODUCTION

Rapeseed (*Brassica napus* L.) is cultivated in a large scale approximately, 20.000 ha in Mazandaran province in the north of Iran. Phoma blackleg (*Leptosphaeria biglobosa*), pathogenicity group 1 (PG-1) or non-aggressive type, has been reported on rapeseed from Golestan province (1). Recently, a typical symptom of rapeseed blackleg has been observed in the regions with a long history of cultivation (Dasht-E- Naz).

MATERIALS AND METHODS

In September 2008, Ascospores of *Leptosphaeria maculans* was obtained from infected stubble residues of commercial Rapeseed Hybrid (Hyola 401)(2). These spores were cultured in V8 medium. Thirteen isolates of *L.maculans* were used for determining Pathogenicity groups (PG) according to the phenotypic interaction (PI) on 7-day old rapeseed cultivars; Glacier, Westar and Quinta. After 10 days, disease severity was rated on a 0–9 scale (3). Wounded cotyledons were inoculated with 10µl of conidial suspensions at 2×10⁷ spores per ml. All plants were maintained in a growth chamber at 21°C (light) to 16°C (dark), with a 16-h photoperiod and relative humidity of 95%. The test was repeated three times.

RESULTS

Two isolates (Es-3 and ES- 12) were classified as belonging PGT, 2 (Es-5 and ES-7) as PG2 and 9 isolates as PG1. PGT isolates gave PI reactions 3 to 4, 7 to 9 and 7 to 9 on Glacier, Quinta and Westar, respectively. As our survey, this is the first report of the occurrence of *Leptosphaeria maculans* PGT in Iran(Figure 1).

DISCUSSION

In some regions in the north of IRAN, the leaf symptoms have been observed as circle lesions with chlorotic border and gray color in center, sometimes lesions separate from leaf. The spots on stems are more elongate and often surrounded by a purple or black border. With attention to high sensitivity of current cultivar of Mazandaran province (Hyola 401) to PG2 and PGT of *L.maculans*, It is possible that to be epidemic of blackleg disease in the case of suitable climatic condition, in future.

ACKNOWLEDGEMENTS

Special thanks to the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) for providing the seeds needed for this testing.

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Figure 1. Phenotypic interaction (PI) of *Leptosphaeria maculans* isolates on five rapeseed cultivars after 10 days.

19 In vitro study on the effect of NanoSilver (Nanosid) on *Sclerotinia sclerotiorum* fungi the causal agent of rapeseed white stem rot

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INTRODUCTION

White stem rot of rapeseed (*Brassica napus* L.) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a serious disease in many areas in the world. This fungus attacks over 360 species of plants (2). It has been reported in Golestan and Mazandaran provinces in north of Iran (1). Chemical spraying is the simplest option for controlling sclerotinia disease in rapeseed fields however many surveys have been done for potential on bicontrol methods and new fungicide applications.

MATERIALS AND METHODS

In this study, the in vitro effectiveness of nanosilver different doses consist of 5, 10, 30, 50, 100,120, 130 and 150 ppm were tested against one isolate of *S. sclerotiorum*. Nanosid solution was incorporated into potato dextrose agar (PDA) medium, after autoclaving. Sclerotia of *S. sclerotiorum* were positioned in the center of Petri dishes containing PDA plus nanosid and were maintained in 25 centigrade. Fungal growth characteristics were determined after 8 and 14 days at all of the treatments.

RESULTS

After 8 days, there was no mycelia growth or sclerotia production in doses more than 10 ppm, while there was observed a significant difference between the mycelia growth of control (Sterile water), 5 and 10 ppm doses of nanosilver. Average per cent of inhabitation effect of nanosilver doses (8 doses) and control treatment on sclerotia were estimated after 14 days respectively 0, 25.33%, 48.17%, 53.33%, 56%, 56%, 57.66%, 61% and also 0 for control (Table 1). After 14 days, a few numbers of new sclerotia were observed in doses of more than 10 ppm (2 to 4 sclerotia) in comparison white 5ppm and control (18–22 sclerotia). Obtained results showed that Nanosilver has fungistatic action and could be used for decreasing sclerotia production and mycelia growth (Fig.1)

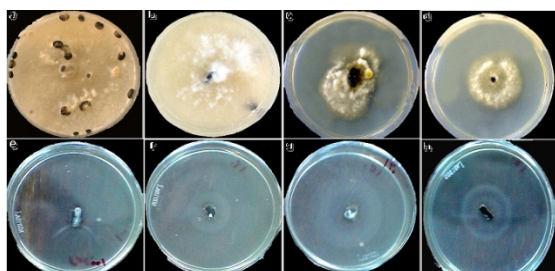


Figure 1. Effect of NanoSilver doses on radial growth of *S.sclerotiorum* after 14 days: a) Control and 5ppm, b) 10 ppm, c)30 ppm and d)50ppm, after 8 days: e)100ppm, f)120 ppm, g)130 ppm and h)150 ppm.

Table 1. Comparison of means for effect of Nanosilver doses on sclerotia of *S.sclerotiorum* after 8 and 14 days.

Nanosilver(ppm)	Mean of square	
	after8days	after14days
Control	75a ²	75a ²
5	31.37b	75a
10	16.75c	56b
30	0d	38.87c
50	0d	35cd
100	0d	33cd
120	0d	33cd
130	0d	31.75cd
150	0d	29.25cd

²Within columns, numbers followed by a common letter are not significantly different ($P=0.05$) according to Duncan's multiple range test.

DISCUSSION

Practical Tests of Nanosid compounds will be done in field condition in future.

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20 Study on the effect of number of spraying with fungicides on rapeseed sclerotinia stem rot control

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INTRODUCTION

Sclerotinia stem rot disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary fungus, is one of the rapeseed important diseases in the world and also in the north of Iran (1). Germination of fungus sclerotia produce apothecia on the soil and the ascospores that released from apothecia can infect rapeseed plants at the flowering stage. Spraying with fungicide in this stage protect the plant from infection.

MATERIALS AND METHODS

In order to study the effect of number of spraying with fungicides against rapeseed sclerotinia stem rot disease caused by *S. sclerotiorum*, an experiment was conducted in complete randomised block design with 3 replications and 7 treatments in Mazandaran province for two years (2006–2007). An infected field (with many sclerotia in the soil) was selected and the rapeseed hybrid Hyola401 planted in it. For disease control at the flowering stage of rapeseed one or twice spraying with fungicides carbendazim (WP60%) and tebuconazole (EC25%) was done. The first spraying was done at the 20%–30% of flowering stage and the second one 14 days after that. In both two years the period of fungi apothecia appearance and ascospores releasing had been started before spraying. The percentage of disease infection on leaves and stems was determined. The disease severity on the plants was scored from 1(lowest infection) to 9(highest infection) and determined in different treatments.

RESULTS

Results of variances analysis and comparison between treatments means by Duncan's test indicated that in first year sprayed treatments compared to control had lower infection and higher yield, but there were not significant differences between one and twice sprayed treatments. In second year and two years combined analysis, control had highest infection and lowest yield and twice sprayed treatments had lowest infection and highest yield.

Table1. Comparison of leaf and stem infection percentage, disease severity and yield between different treatments in two years.

Treatments	%leaf infection	%stem infection	Disease severity	Yield Kg/ha
S	55a ^z	49a ^z	6.6a ^z	3208d ^z
C	15bc	11bc	3.2c	3793bc
CC	3.5d	0.5d	0.7f	4020a
F	21b	15b	4.1b	3720c
FF	8.5cd	5.5cd	2.4d	3975a
FC	4d	1d	0.7f	4003a
CF	7.5cd	3.5d	1.6e	3921ab

Control(S), Carbendazim(C), Twice carbendazim (CC), folicur(F), Twice folicur (FF), Folicur at first and carbendazim at second application(FC), Carbendazim at first and folicur at second application (CF)

^zWithin columns, numbers followed by a common letter are not significantly different ($P=0.01$) according to Duncan's multiple range test.

DISCUSSION

Chemical control of rapeseed sclerotinia stem rot is an effective method in the north of Iran. Depending on the weather condition and field growth, one or twice fungicide application is needed. For best disease control we recommend the disease managing by integration of different methods like mechanical, agronomical, biological and chemical.

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98 The biology and management of chestnut rot in south-eastern Australia

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INTRODUCTION

The Australian chestnut industry is currently small, but has potential to expand due to increasing local and export demand. The European chestnut, *Castanea sativa* is the main species grown in Australia today. Internal rot of chestnuts is a significant problem facing the industry and is what this study will be investigating. Internal rot affects the kernel, manifesting in light, medium and/or dark brown lesions occurring on the endosperm, and embryo. It is often not visible externally and is only realised when the nut is opened. The incidence of chestnut rot at Melbourne markets has been recorded as high as 40% (1). Consumer rejection of the chestnuts is highly likely with rot incidence at these levels. Without an appropriate control strategy, the Australian chestnut industry is unlikely to grow.

The main cause of chestnut rot is reported to be endophytic, caused by the fungal Ascomycete *Phomopsis castanea* (Sacc.) Höhn (1,3). Research also shows that infection by ascospores during flowering is a mode of infection (2).

The aims of this study are to survey the incidence of chestnut rot in south eastern Australia, New South Wales (NSW) and Victoria (VIC), identify the pathogen responsible for causing chestnut rot, and to study the disease cycle.

MATERIALS AND METHODS

Field and market surveys. A hierarchical sampling strategy was used. Twenty two orchards were sampled across VIC and NSW. Orchards were classed into 6 regions based on geographical distance i.e. 100km proximity (see fig 1 for orchard locations). Four commercial varieties were assessed for rot incidence: Buffalo Queen, Red Spanish, Purton's Pride, and Decoppi Marone (300 nuts/orchard). Nuts were dissected and visually assessed for rot.

Flemington Markets in NSW were also sampled for chestnut rot. Nuts sourced from 5 orchards were sampled, 300 nuts/orchard. Decoppi Marone was surveyed from 2 orchards and Purton's Pride from 3 orchards.

Pathogen identification. Diseased chestnuts from 12 of the surveyed farms in VIC and NSW had the pathogen isolated from them using standard isolating protocols (1, 3). The pathogen was then identified morphologically.

Observation of the teleomorph was completed through microscopic observation of 30 decaying burrs from an orchard in Mullion Creek, NSW.

Endophyte studies. Samples were collected from healthy floral and vegetative tissues at Mullion Creek NSW. Seventy-five samples per tissue type were tested.

RESULTS AND DISCUSSION

Field Survey

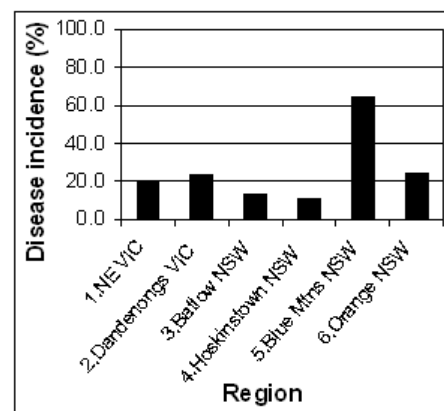


Figure 1. Incidence of chestnut rot from nuts sampled field survey across 6 regions in NSW and VIC. Total number of nuts in survey =6600.

Disease incidence of chestnuts tested from Flemington markets, NSW was found to be $\leq 10\%$ for all orchards and varieties tested. This value is within the acceptable level of rot set by the Australian chestnut industry.

Pathogen identification. Of the 568 isolates obtained, 62% of were identified as *Phomopsis castanea*. The teleomorph of *Diaporthe castaneti* Nitschke. was observed growing on decaying burr tissue. This indicates that aerosols of ascospores are a source of infection in the disease cycle.

Endophyte studies. Tissues displaying the highest rates of pathogen isolation include female flowers (82%), male flowers (59%), pedicel (28%), leaf margin (33%), and 1st year growth (17%). Low isolation levels were recorded in petioles (9%), and leaf mid veins (9%), second year stems (8%), and third and fourth year bark (3%). The pathogen was not recorded in third and fourth year xylem tissue, indicating pathogen travel via xylem tissue is not significant and likely not systemic.

CONCLUSION

Disease incidence varies greatly between regions. This is likely due to factors including regional and micro-climates, rainfall during flowering, the susceptibility of host variety to the pathogen, and the virulence of the pathogen.

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