

AUSTRALASIAN PLANT PATHOLOGY SOCIETY

Conference

14-16 September 2015

FREMANTLE • WESTERN AUSTRALIA



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Welcome

Welcome to Fremantle for the 20th Biennial Conference of the Australasian Plant Pathology Society.

Fremantle (population 27,000) located at the mouth of the Swan River is a major port city servicing the State of Western Australia. The city is named after Captain Charles Fremantle, the English naval officer who had pronounced possession of Western Australia and who established a camp at the site. Fremantle is renowned for its well-preserved architectural heritage, including convict-built colonial-era buildings, an old jetty and port and prisons; presenting a variety and unity of historic buildings and streetscapes. The city also hosts the campus of the University of Notre Dame Australia and is home to the Fremantle (Dockers) Football Club.

The conference will be held over three days at the Esplanade Hotel and we are confident that with the program of events and presentations that you will have an exciting three days.

The conference logo

Given the location of the conference in the maritime location of Fremantle for might be forgiven for thinking that the logo is seaweed. In fact it is the Western Australian Christmas tree. It's botanical name is *Nutysia floribunda* and it's a parasitic plant indigenous to Western Australia.

Philip O'Brien
APPS 2015 Conference Convenor

Conference Organising Committee

Philip O'Brien, Murdoch University—Conference Convenor
Treena Burgess, Murdoch University—Scientific Programme
Kith Jayasena, Dept Agriculture Fisheries & Food WA—Workshops
Elaine Davison, Curtin University—Secretary
Brenda Coutts, Dept Agriculture Fisheries & Food WA—Virology Workshop
Vera Andjic, Dept Agriculture Fisheries & Food WA—Committee Member
Kirsty Bayliss, Murdoch University—Committee Member
Monica Kehoe, Dept Agriculture Fisheries & Food WA—Committee Member
Judith Lichtenzweig, Curtin University—Committee Member
Aaron Maxwell, Dept Agriculture Fisheries & Food WA—Committee Member
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Sponsors

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

Gold sponsor



The GRDC's mission is to invest in research and development for the greatest benefit to its stakeholders—grain growers and the Australian Government. The Corporation links innovative research with industry needs. The GRDC's vision is for a profitable, internationally competitive and ecologically sustainable grains industry.

The GRDC's research portfolio covers 25 leviable crops spanning temperate and tropical cereals, oilseeds and pulses, worth over \$7 billion a year in farm production, alone. The GRDC is a statutory corporation, operating as a research investment body in partnership with growers and Government.

Funding is provided through a levy on grain growers. This is determined each year by the grains industry's peak body. The Australian Government matches this funding, up to an agreed ceiling. For more information please see our website: <http://www.grdc.com.au/>

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Curtin University's Centre for Crop and Disease Management (CCDM) is delivering high-impact research that makes a real difference to agricultural industries. Supported by the Grains Research and Development Corporation (GRDC), our centre aims to reduce the impact of major crop diseases through genetics, breeding and fungicide research, while improving agronomy and farm management practices.

Lanyard sponsor



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.

Exhibitors

CONVIRON



Conviron is the world leader in the design, manufacture and installation of controlled environment systems for plant science research.

Conviron's reach-in plant growth chambers, walk-in rooms and Argus Control Systems provide precise, uniform, and repeatable control of temperature, light, humidity, dehumidification, CO₂, and other environmental conditions. Learn more at www.conviron.com.au.

CCDM



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Australasian Plant Pathology Society



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Delegate information

Registration desk

The registration desk will be located in the Southern Cross Lobby of the Esplanade Hotel Fremantle and will be open as follows:

Sunday 13 September	1800–1930 (Welcome Reception, WA Maritime Museum)
Monday 14 September	0700–1745
Tuesday 15 September	0700–1735
Wednesday 16 September	0800–1720

Please visit the registration desk upon arrival at the conference to collect your name badge and handbook.

The registration desk can be contacted during the above hours via 0434 032 683.

Please direct any questions you may have regarding registration, attendance, accommodation or social functions to the staff at this desk.

Catering

Morning tea, afternoon tea and lunches will be held in the Indian Ocean Suite. Lunches will be served as an informal stand-up buffet. Dietary requirements noted on your registration form have been passed on to catering staff and will be available from a dedicated catering station. Please see a member of the banquet staff if you require assistance.

Mobile phones

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

Name badges

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

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.

Participant list

The delegate list was emailed to all registered attendees. Delegates who have indicated on their registration form that they do not want their name, organisation and state to appear on the delegate list have not been included.

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 at the registration desk for any late changes to sessions.

Speakers' preparation room

A speakers' preparation room will be open during the following hours:

Monday 14 September	0800–1730
Tuesday 15 September	0800–1730
Wednesday 16 September	0800–1600

All speakers must take their presentation to the speakers' preparation room 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 audio visual equipment.

Useful telephone numbers

Swan Taxis	13 13 30
Black and White Taxis	13 10 08
Tricolour Taxis/Coastal Taxis	13 22 27
Uber	www.uber.com

Qantas	13 13 13
Virgin Blue	13 67 89
Jetstar	13 15 38

Public transport

Delegates may also take the Connect Bus from the airport to the Perth CBD station, and then take the train to Fremantle. You'll find the Connect Bus shuttle stops directly outside Terminal 3. There are five pick-up and drop-off locations across the city with tickets costing \$15 one way per person.

Please visit perthairportconnect.com.au or call 08 9277 4666 for full information on stops and timetables. Only weekends and public holidays, the last Connect Bus service from the airport departs at 2230 hrs.

To catch the train to Fremantle, depart the Connect Bus service at the WA Museum, Beaufort Street. From here it is short walk to the Central Perth Train Station. The train trip to Fremantle takes approximately 30 minutes and costs \$4.50 one way per person.

Transperth buses (routes 37 and 40) also run from the airport to the city, departing from outside Terminal 3. The Transperth bus routes are operated solely by low floor, accessible buses with a ramp that can be lowered on request. An indicative timetable for route 37 may be found [here](#), and [here](#) for route 40. A one-way ticket costs \$4.20 per person.

For more information on buses and trains in and around Perth/Fremantle, please visit www.transperth.wa.gov.au.

Social program

Welcome reception

Sunday 13 September 2015
1800–1930

Western Australian Maritime Museum, Victoria Quay, Fremantle WA

Included with full registrations. Additional tickets may be purchased for \$60 (including GST). The WA Maritime Museum is a 10-minute walk from The Esplanade Hotel Fremantle.

Conference dinner

Tuesday 15 September 2015
1830–1130

Fremantle Prison, 1 The Terrace, Fremantle WA

Included with full registrations. Additional tickets may be purchased for \$120 (including GST). The Fremantle Prison is a nine-minute walk from The Esplanade Hotel Fremantle.

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 1984, Stanislaus Fish 1976).

In 2011, the McAlpine Lecture will be delivered by Professor Lester Burgess from the University of Sydney.

Previous McAlpine Lecturers

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

2011 Honorary Professor Lester Burgess, University of Sydney, NSW
A love affair with Fusarium

2013 Dr Shaun Pennycook, Landcare Research NZ
Fungal Names in Flux

McAlpine lecturer 2015: Professor Giles Hardy

From then till now; 25 years of research into the biology and management of Phytophthora cinnamoni

Professor Giles Hardy has made significant contributions to our understanding about the biology, pathology and control of *Phytophthora* species in natural ecosystems, rehabilitated mines and horticulture. In particular his research has contributed greatly to the existing knowledge on how, when, at what rates to apply the fungicide and priming agent phosphite to natural plant communities, this fungicide is used worldwide to treat natural ecosystems as a result. More recently, Giles has started to look closely at how plant diseases impact on forest and woodland ecosystem function and health and his research activities have extended into projects that examine the impact of plant disease on fauna and on water. He has also worked on other diseases of trees in plantations and natural ecosystems. Giles has instigated and directed two researcher centres; the Centre for Phytophthora Science and Management and the multidisciplinary State Centre of Excellence on Climate Change and Woodland and Forest Health. Giles is an enthusiastic and dedicated educator and has supervised and mentored numerous PhD students and early career researchers. His outreach goes far beyond scientific publications as he engages widely within the community. All in all, Giles is enthusiastic, an excellent communicator and a strategic thinker and these attributes have enabled him to build an outstanding scientific career.

Allen Kerr Postgraduate Prize

The Allen Kerr Postgraduate Prize commemorates the significant contribution to research in plant pathology made by Professor Allen Kerr AO, recipient of the inaugural Australia Prize. The award is made at the APPS biennial conference and is open to postgraduate student members of APPS undertaking original research relevant to Australasia. The prize is awarded by the Society for the best piece of original research by a postgraduate student in the field of plant pathology. The prize is normally awarded on the basis of publication in refereed journals.

Keynote biographies

Dr Pierre Abad, Senior Scientist at INRA

Pierre Abad is senior Scientist at INRA. He earned his Ph. degree in plant pathology from the University of Paris XI Orsay in 1986. Head of the Sophia Agrobiotech Institute in Sophia Antipolis (France,) he coordinates the Plant-Nematode Interaction team which develops researches on four different areas (i) Plant response during compatible interaction, (ii) Nematode parasitism genes analysis, (iii) Structure and evolution of the genome (iv) Evolution of nematode pathogenicity and durability of plant resistance genes. He initiated and coordinated the sequencing project of the root knot nematode genome *Meloidogyne incognita* which was a double premiere: first genome of plant parasitic animal and first genome of a parthenogenetic animal.

Dr Fen Beed, Regional Director for East and Southeast Asia, AVRDC

Dr Fen Beed, Regional Director for East and Southeast Asia, AVRDC - The World Vegetable Center, has led research and development activities in both temperate and tropical climates targeting a diversity of pathogens and crops. Prior to joining AVRDC Fen worked for 14 years in Africa as Senior Plant Pathologist for IITA, based in Benin, Uganda and then Tanzania. Studies focused on surveillance, diagnostics, epidemiology, disease management, risk assessment, mycotoxins, microbial genetic resources for agriculture, and biological control of weeds. Between 1994 and 2000, he worked at the University of Nottingham, UK and simultaneously served as a consultant for ADAS, studying interactions between crop physiology, pathogen epidemiology and the influence of environmental conditions in the field. Prior to 1994 Fen worked and studied at the Universities of Sheffield, Strathclyde and University College London, UK where his research focused on biochemical interactions between plant hosts and their fungal pathogens. Current drive of Fen's work is to coordinate activities, including IPM, to increase production and consumption of affordable, safe and nutritious, health promoting vegetables.

Prof Richard Hamelin, Senior Research Scientist, Natural Resources Canada and Professor, Faculty of Forestry, University of British Columbia

Dr Richard Hamelin obtained a BSc. from McGill University in 1982, a Master's of Pest Management from Simon Fraser University in 1986 and a PhD from the University of Kentucky in 1990. He has 30 years of experience in forest health research has published over 100 peer-reviewed scientific papers in forest pathology, fungal population genetics and fungal genomics. He has been president of the Canadian Phytopathological Society and the Quebec Society for Plant Protection and has received the IUFRO Scientific Achievement Award (2014), the Queen Elizabeth II Diamond Jubilee award (2012) as well as Merit Awards from Natural Resources Canada (2008), the Canadian Forest service (2008), the Canadian Food Inspection Agency (2007), and the Quebec Society for Plant Protection (2008) for his work on the application of genomics in forest pathology.

Dr Steven Lindow, Executive Associate Dean, College of Natural Resources, University of California Berkeley

Our research group studies aspects of epiphytic bacteria that live on healthy plants' surfaces, emphasizing bacteria active in ice nucleation, causing frost damage to plants. We also study plant pathogenic bacteria that inhabit plant surfaces before infection. We use molecular genetic and ecological approaches to study how epiphytic bacteria interact with other microorganisms on plants, and with the plants on which they live. We seek to better understand adaptations epiphytic bacteria have evolved to exploit this unique habitat

Dr Arvind Varsani, University of Canterbury, New Zealand and University of Cape Town, South Africa

Arvind Varsani is a molecular virologist studying viral evolution through recombination and global viral movement patterns of geminiviruses and circoviruses. Over the last five years he has been pioneering new approaches to studying viruses, particularly circular DNA viruses in various ecosystems, both terrestrial and aquatic. Some of these approaches are proving to be ideal for viral surveillance.

Associate Professor Carolyn Young, The Samuel Roberts Noble Foundation, Ardmore, Oklahoma

Dr Young joined the Forage Improvement Division at the Samuel Roberts Noble Foundation (Ardmore, Oklahoma) in 2006 to lead a research program on endophytes in cool season grasses. She also runs a program on *Phymatotrichopsis omnivora*, a devastating root pathogen endemic to the south-western USA. Dr Young completed her studies at Massey University, in Palmerston North, New Zealand where she earned her BSc (1993), MSc (1999) and PhD (2005) in molecular biology from the Institute of Molecular BioSciences.

The Young lab addresses the utilization of *Epichloë* species as value added symbionts to benefit the production of forage grasses. These fungi are well known for the production of secondary metabolites that provide protection to their hosts. We are interested in endophyte discovery and exploring the diversity of these symbionts, among and between host species. Dr Young has developed molecular tools to rapidly screen large grass populations to define the endophyte diversity within. Combining this with genomics we are able to unravel the life history strategy and describe the evolutionary history of a given isolate. Of particular interest are hybrid *Epichloë* species with common progenitors that have arisen from independent hybridisation events. In addition, Dr Young works with grass breeders and agronomists to develop endophyte-infected tall fescue cultivars. This work combines development of elite grass cultivars with *Epichloë* isolates known to aid with host persistence.

Program

SUNDAY 13 SEPTEMBER 2015

1800–1930	WELCOME RECEPTION	WA Maritime Museum
1930–2100	Council of the Society meeting	Esplanade Hotel

MONDAY 14 SEPTEMBER 2015

0700	Management Committee breakfast	Orion Room	
0800–1730	Registration open	Southern Cross Lobby	
0830–0900	Opening Ceremony Welcome by Convenor, Acknowledgement of Country and Housekeeping Chief Scientist Premier’s representative— D Faragher Introduction of keynote speaker	Sirius Room	
0900–0945	PLENARY 1 <i>The making of tree pathogens: a genomic dissection</i> Dr Richard Hamelin , University of British Columbia	Sirius Room	
0945–1015	MORNING TEA	Indian Ocean Suite	
1015–1240	CONCURRENT SESSION 1		
	1a BIOLOGY: plant pathogen interactions	1b MANAGEMENT: chemical control	
	1c BIOLOGY: genetics		
	Sirius Room	Pleiades Room	
	Orion Room		
1015	<i>Multi-function AVE1-like effectors in scab pathogens</i> Kim Plummer	<i>Persistence of fungicide efficacy on mandarin fruit</i> Andrew Miles	<i>Transcriptome of the foliar gymnosperm pathogen Dothistroma septosporum</i> Rosie Bradshaw
1045	<i>Comparative analyses used to investigate host-specificity determinants in Venturia pirina and V. nashicola</i> Shakira Johnson	<i>Foliar phosphite application has minor phytotoxic impacts across a diverse range of conifers and woody angiosperms</i> Peter Scott	<i>Genetic variation among Australian isolates of Fusarium oxysporum from strawberry</i> Michelle Paynter
1105	<i>The secretome of Pyrenophora teres f. teres: role of virulence-associated proteins during net blotch disease of barley</i> Amanda Able	<i>Resistance of Erysiphe necator to QoIs and DMIs</i> Suzanne McKay	<i>Characterisation of rice blast races in Australia</i> Xiangling Fang
1125	<i>Eucalyptus grandis defence responses against the myrtle rust pathogen, Puccinia psidii—insights from RNA-SEQ transcriptome profiling</i> Louise Shuey	<i>Efficacy of soil amendment to control verticillium wilt disease</i> Veradina Dharjono	<i>The use of next-generation sequencing to identify sequence variation in pathogenicity-associated genes of Fusarium oxysporum f.sp. cubense</i> Elizabeth Czislowski
1145	<i>Transcription regulators of aphid resistance in Medicago truncatula</i> Lingling Gao	<i>Phosphite for control of kauri dieback</i> Ian Horner	<i>Unisexual mating in Huntiella moniliformis</i> Brenda Wingfield
1205	<i>High coverage in planta RNA sequencing identifies Fusarium oxysporum effectors and Medicago truncatula resistance mechanisms</i> Louise Thatcher	<i>Postharvest treatment of taro corms to minimise biosecurity risks from plant-parasitic nematodes</i> Sunil Singh	Lightning presentations DISEASE MANAGEMENT: chemical control <i>Efficacy of chlorine, chlorine dioxide and ultraviolet radiation as disinfectants against waterborne pathogens in irrigation water (Poster 47)</i> Kelly Scarlett

1225	Lightning presentations DISEASE BIOLOGY: pathogen genetics <i>Molecular and pathogenic structure of the current Australian Ascochyta rabiei population</i> (Poster 37) Yasir Mehmood <i>Secondary metabolome of the forest pathogen Dothistroma septosporum</i> (Poster 41) Ibrahim Kutay Ozturk <i>The genetic basis of sensitivity of barley to toxins produced by the net blotch pathogen Pyrenophora teres f. teres</i> Amanda Able <i>Population structure and infection biology of Phytophthora pluvialis in Pinus radiata forests in New Zealand</i> (Poster 8) Simren Brar	Lightning presentations DISEASE BIOLOGY: plant/pathogen interactions <i>Application of biotechnology for nematode control in crop plants</i> (Poster 17) John Fosu-Nyarko <i>Biotic and abiotic factors predisposing marri (Corymbia calophylla) to canker disease caused by Quambalaria coyrecup in the southwest of Western Australia</i> (Poster 46) Sarah Sapsford <i>Getting to the root of the problem: Rhizoctonia solani in Champasak, Lao PDR</i> (Poster 9) Lester Burgess <i>Analysis of EguCAD1, an oil palm lignin biosynthesis homologue</i> (Poster 41) Agnieszka Mudge	<i>Interaction of fungicide and genetic resistance for the management of barley leaf rust</i> (Poster 27) Kithsiri Jayasena <i>Sensitivities of New Zealand isolates of Zymoseptoria tritici to succinate-dehydrogenase-inhibiting (SDHI) fungicides</i> (Poster 35) Virginia Marroni <i>Control of foliar diseases in Australian strawberry runner nurseries</i> (Poster 20) Apollo Gomez <i>Azoxystrobin offers potential for chemical control of rice blast (Magnaporthe oryzae) in Australia</i> (Poster 43) Dolar Pak <i>Down-regulation of the Glucan synthase-like 6 gene (HvGsl6) in barley leads to decreased callose accumulation and increased susceptibility against Blumeria graminis f. sp. Hordei</i> (Poster 10) Jamil Chowdhury <i>High incidence of cereal viruses in Victorian crops</i> (Poster 64) Linda Zheng <i>A modified method for Pyrenophora teres F. maculata spore production</i> (Poster 51) Luise Sigel <i>The crown rot deadhead phenomenon in durum wheat</i> (Poster 30) Noel Knight <i>Viability of plant pathogen fungal spores over time at different temperatures carried by tested materials and their relative abilities and effectiveness as spore carriers</i> (Poster 5) Papori Barua <i>The role of woodborers on the spread and infection of Ophiostomatoid fungi in marri and jarrah</i> (Poster 49) Stephen Seaton <i>Understanding the barley leaf rust new pathotype 5457 P- in Western Australia</i> (Poster 28) Kithsiri Jayasena
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1240–1400	LUNCH AND POSTER VIEWING Student Lunch Editors Lunch	Indian Ocean Suite Pleiades Room Orion Room
1400–1445	PLENARY 2 <i>Linking microbial ecology with plant pathology to achieve disease control and increase plant productivity</i> Professor Steven Lindow, University of California, Berkeley	Sirius Room
1445–1515	AFTERNOON TEA	Indian Ocean Suite

	2a BIOLOGY: plant pathogen interactions	2b MANAGEMENT: biological control	2c BIOLOGY: pathogenicity
	<i>Sirius Room</i>	<i>Pleiades Room</i>	<i>Orion Room</i>
1515	Quantitative PCR (qPCR) and mid-infrared spectroscopic (MIR) analyses of powdery mildew on grape bunches Eileen Scott	Efficacy of conventional and biological fungicides for managing powdery mildew on two muskmelon varieties with different genetic susceptibility to the disease Michael Matheron	Pathogenicity determinants and high level of variation in the multinucleate genome of a single <i>Rhizoctonia solani</i> AG8 isolate Jonathan Anderson
1545	Early defence responses in <i>Elaeis guineensis</i> lignin biosynthesis pathway during pathogenesis of <i>Ganoderma boninense</i> Carmen Goh	The effects of a novel Chrysovirus on asexual and sexual spore production in <i>Aspergillus</i> species Michael Pearson	The impact of root diseases on subterranean clover pastures across Australia Kevin Foster
1605	Differential accumulation of callose, arabinoxylan and cellulose in non-penetrated versus penetrated papillae on leaves of barley infected with <i>Blumeria graminis</i> f. sp. <i>hordei</i> Alan Little	Improving soil organic carbon levels enhances suppression of root lesion nematode in sugarcane soils Graham Stirling	Investigating soilborne necrotrophic fungi associated with black root rot in avocado Roger Shivas
1625	Neuronal signalling molecules as targets for green peach aphid (<i>Myzus persicae</i>) control via RNA interference Sharmin Rahman	Integrated treatment of citrus fruit for the control of <i>Penicillium digitatum</i> , causal agent of citrus green mould: a revision of common praxis Iona Basdew	Characterisation of the blueberry stem blight pathogen in northern NSW Chris Rothwell
1645	Endophytic <i>Trichoderma</i> in the disease management of <i>Ganoderma</i> disease of oil palm—deciphering the mechanisms Shamala Sundram	Suppression of <i>Fusarium</i> crown rot in wheat by endophytic actinobacteria Margaret Roper	Investigating the pathogenicity of <i>Neocosmospora</i> species on germinating chickpea seedlings under controlled conditions Kylie Wenham
1705	Histopathology of <i>Stagonosporopsis tanacetii</i> infected pyrethrum seed Md Abdullahil Baki Bhuiyan	Biological control of <i>Monilinia</i> and <i>Botrytis</i> in Tasmanian cherries Dean Metcalf	The impact of root-lesion nematodes, <i>Pratylenchus</i> spp., on summer grain crops Kirsty Owen
1725		Novel green manure: control of specific replant disease on the rosaceous ornamental tree <i>Sorbus aucuparia</i> Julia Lock	
1745	Close of Day One		
1830	REGIONAL COUNCILLORS DINNER (informal)		TBC

TUESDAY 15 SEPTEMBER 2015

0700–0800	Presidents breakfast	Orion Room
0800–1700	Registration open	Southern Cross Lobby
0830–0915	Presidential Address	Sirius Room
0915–1000	PLENARY 3	Sirius Room

Plant root knot nematode interaction: a sophisticated dialogue

Dr Pierre Abad, INRA

1000–1030	MORNING TEA	Indian Ocean Suite
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1030–1300	CONCURRENT SESSION 3	
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	3a DIAGNOSTICS: phylogenetics	3b DISEASE AND THE COMMUNITY	3c BIOLOGY: pathogenicity
	Sirius Room	Pleiades Room	Orion Room
1030	<i>Cercosporoid tree of life</i> Pedro Crous	<i>A community 'Dieback Information Delivery Management System'—DIDMS</i> Tilo Massenbauer	<i>Impact of the foliar pathogen <i>Phytophthora pluvialis</i> and soil borne pathogen <i>P. cinnamomi</i> on <i>Pinus radiata</i> root development in controlled conditions</i> Peter Scott
1100	<i>Unraveling the taxonomy of the <i>Colletotrichum</i> species causing anthracnose in chili in Australia and SE Asia</i> Dilani De Silva	<i>Utilising citizen science to monitor <i>Corymbia calophylla</i> (Marri) canker incidence and severity</i> Alex Chapman	<i>Age-related susceptibility of <i>Eucalyptus</i> species to <i>Phytophthora boodjera</i> prov. Nom.</i> Agnes Simamora
1120	<i>Characterisation of <i>Diaporthe</i> species complex on soybean</i> Kristina Petrovic	<i>Learning and teaching academic standards statement for agriculture—how does plant pathology fit?</i> Amanda Able	<i>Foliar inoculation of <i>Agathis australis</i> infected with the soil borne pathogen <i>Phytophthora agathidicida</i></i> Echo Herewini
1140	<i><i>Colletotrichum</i> species associated with citrus anthracnose in Tunisia</i> Paul Taylor	<i>Holy mole! Grainman (Batman)! What's that? Can our farmers and agronomists recognise pests and diseases in grain crops?</i> Dominie Wright	<i>Variation between isolates of <i>Neofusicoccum</i> species in pathogenicity and sporulation on grapevine shoots</i> Amna Shafi
1200	<i>Molecular phylogenetic analysis of Australian <i>Bipolaris</i> isolates reveals novel taxa and host records</i> Yu Pei Tan	<i>Experience of a lifetime—plant pathology and IDM in Laos</i> Kylie Ireland	<i><i>Agrobacterium</i>-mediated transformation of <i>Ganoderma boninense</i> to discern early stage pathogenesis of basal stem rot</i> Nisha Govender
1220	<i><i>Lasiodiplodia</i> (Botryosphaeriaceae) associated with Myrtales from Southern Africa</i> Lucas Shuttleworth	<i>The plant health RD&E investment plan for the Australian vegetable industry</i> Doris Blaesing	<i>Does <i>Phytophthora</i> predispose Marri (<i>Corymbia calophylla</i>) to <i>Quambalaria coyrecup</i> canker?</i> Louise Croeser
1240	<i>Phylogenetic relationships and population diversity of commonly found phytoplasma strains in Sri Lanka</i> Chamini Kanatiwela De Silva		Lightning presentations DISEASE BIOLOGY: Pathogenicity <i>Postharvest diseases of mango in Fiji (Poster 33)</i> Mereia Fong Lomavatu <i>Are wounds necessary for <i>Botryosphaeria</i> infections in blueberries? (Poster 54)</i> Shanika Tennakoon

Host range of Neocosmospora species causing root rot of legumes in Australia (Poster 59)

Kylie Wenham

Can spectral measurements be used to detect Phytophthora-related stress in Corymbia calophylla (marri)? (Poster 13)

Louise Croeser

1300–1400	LUNCH AND POSTER VIEWING			Indian Ocean Suite
1400–1445	PLENARY 4 <i>Fungi impacting forage production: fungal friend and fungal foe</i> Dr Carolyn Young , The Samuel Roberts Noble Foundation			Sirius Room
1445–1515	AFTERNOON TEA			Indian Ocean Suite
1515–1635	CONCURRENT SESSION 4			
	4a PREPARDNESS: Biosecurity	4b SURVEILLANCE: monitoring	4c MANAGEMENT: resistance	
	Sirius Room	Pleiades Room	Orion Room	
1515	<i>Safeguarding cultural and functional integrity of forests by harmonising cultural and scientific values</i> Stanley Bellgard	<i>Spore trapping methods for the early detection of invasive forest pathogens</i> Anna Hopkins	<i>RNA silencing of RNAi effectors reduces root-knot nematode infection</i> Sadia Iqbal	
1535	<i>Predicting high-impact pests and pathogens of plants</i> Kylie Ireland	<i>Corymbia calophylla (Marri) canker disease incidence at the temporal and landscape level</i> Trudy Paap	<i>The prevailing pathogen(s) within the black spot disease complex determines expression of host resistance in field pea (Pisum Sativum)</i> Hieu Tran	
1555	<i>Human-mediated spread of plant pests via networks</i> Kirsty Bayliss	<i>Rain splash of fungal spores in tree canopies</i> Monika Walter	<i>A new seedling bioassay technique for screening for resistance to white leaf spot disease</i> Niroshini Gunasinghe	
1615	<i>Mapping rice diseases for targeted deployment of resistant varieties in India</i> Adam Sparks	<i>New oomycete genus and hybrid Phytophthora discovered as part of stream-based catchment surveillance for kauri dieback</i> Stanley Bellgard	<i>Two sides of the coin: investigating defence responses against Phytophthora cinnamomi in Eucalyptus nitens using dual RNA sequencing</i> Louise Shuey	
1635–1735	Biennial General Meeting			Sirius Room
1830	CONFERENCE DINNER			Fremantle Prison

WEDNESDAY 16 SEPTEMBER 2015

0800–1700	Registration open	Southern Cross Lobby
0830–0915	McAlpine Lecture— Dr Giles Hardy	Sirius Room
0915–1000	PLENARY 5 <i>Principals to sustain crop productivity and quality</i> Dr Fen Beed , AVRDC—The World Vegetable Center	Sirius Room

1000–1030	MORNING TEA		Indian Ocean Suite
1030–1300	CONCURRENT SESSION 5		
	5a BIOLOGY: epidemiology	5b MANAGEMENT: resistance	
	Sirius Room	Pleiades Room	
1030	<i>Myrtle rust, impacts on Myrtaceous diversity in Australia</i> Geoffrey Pegg	<i>Plant parasitic nematodes causing strife in Western Australian broadacre crops</i> Sarah Collins	
1100	<i>Injection-infiltration of attached grapefruit with <i>Xanthomonas citri</i> subsp. <i>citri</i> to evaluate seasonal population dynamics in citrus canker lesions</i> Jim Graham	<i>Identification of the first turnip mosaic virus resistance gene in <i>Brassica juncea</i></i> Eviness Nyalugwe	
1120	<i>Microbial and microclimate profiles in almond stockpiles</i> Chin Gouk	<i>Assessing resistance and tolerance of cereal varieties to root lesion nematodes in multi-environment field trials</i> Katherine Linsell	
1140	<i>Studies of sexual reproduction of <i>Phyllosticta citricarpa</i>, the causal agent of citrus black spot</i> Nga Tran	<i>Transcriptional regulation in a maize population segregating for susceptibility to grey leaf spot disease</i> Dave Berger	
1200	<i>Temperature adaptation in isolates of <i>Sclerotinia sclerotiorum</i> infecting <i>Brassica carinata</i></i> Margaret Uloth	<i>An efficient and reliable glasshouse screening method to evaluate bacterial blight resistance in field pea (<i>Pisum sativum</i>)</i> Pragya Kant	
1220	<i>Teratosphaeria pseudoeucalypti, recently detected and seriously damaging red gum eucalypts in Uruguay</i> Carlos A Perez	Lightning presentations <i>Pyramiding yellow spot resistance loci into fixed lines results in genetic gain (Poster 50)</i> Manisha Shankar <i>Yield loss to Fusarium crown rot: is there a better choice among barley and wheat varieties? (Poster 23)</i> Daniel Hüberli <i>Efficacy of selected bactericides in reducing fruit lesions caused by <i>Pseudomonas syringae</i> PV. <i>Syringae</i> (Poster 34)</i> Virginia Marroni	
1240	Lightning presentations DISEASE BIOLOGY: Epidemiology <i>Application of quantitative precipitation model to improve spatial resolution of plant disease forecast models (Poster 29)</i> Hyo-Suk Kim <i>Does soil acidity impact Western Australia's broadacre crops ability to resist root lesion nematodes? (Poster 12)</i> Sarah Collins <i>How root-lesion nematode (<i>Pratylenchus thornei</i>) affects wheat growth and reduces grain yield (Poster 55)</i> John Thompson <i>Seasonality of <i>Phyllosticta</i> spp. in citrus leaf litter (Poster 38)</i> Andrew Miles	Lightning presentations DISEASE MANAGEMENT: Resistance <i>Genetic resistance of <i>Eucalyptus</i> clones to <i>Ceratocystis fimbriata</i> strains (Poster 44)</i> Carlos A. Pérez <i>Resistance-related responses in model and native plant species infected with <i>Phytophthora cinnamomi</i> (Poster 25)</i> Md Tohidul Islam <i>Common bean (<i>Phaseolus vulgaris</i>) seed lustre and country of origin can be indicators of resistance to <i>Pythium hypocotyl</i> disease (Poster 31)</i> Yu Pin Li <i>Are Western Australia's horticultural crops susceptible to southern sting nematode? (Poster 60)</i> Carla Wilkinson	

Host preferences of Pratylenchus quasitereoides—a new challenge in soil-root battlefield (Poster 6)

Farhana Begum

Application of nanotechnology: a field trial to study the feasibility of a non-stick compound in preventing potential spread of Phytophthora infested soil through anthropogenic activities (Poster 18)

Jamba Gyeltshen

1300–1400	LUNCH AND POSTER VIEWING		Indian Ocean Suite
1400–1445	PLENARY 6 <i>The emergence and evolutionary dynamics of maize streak virus</i> Dr Arvind Varsani , University of Canterbury		Sirius Room
1445–1515	AFTERNOON TEA		Indian Ocean Suite
1515–1655	CONCURRENT SESSION 6		
	6a DIAGNOSTICS: identification methods	6b SURVEILLANCE: new reports	6c VIROLOGY
	Sirius Room	Pleiades Room	Orion Room
1515	<i>Multi-locus genome screening of Candidatus liberibacter solanacearum samples indicates limited genetic diversity of the invasive bacterium within New Zealand</i> Grant Smith	<i>Fusarium fujikuroi associated with bakanae disease of rice in southern Lao PDR</i> Lester Burgess	<i>South-west Australia: a global hotspot of virus diversity</i> Stephen Wylie
1535	<i>Can plants tell you if they are infected with viruses?</i> Gardette Valmonte, Robyn MacDiarmid	<i>Prevalence and economic impact of parasitic nematodes of field crops in western Victoria</i> Joshua Fanning	<i>Wind spread of plant viral pathogens into northern Australia</i> Solomon Maina
1555	<i>Uromycladium acaciae is the cause of severe rust symptoms on Acacia mearnsii in South Africa</i> Mike Wingfield	<i>Managing on-farm biosecurity risk through pre-emptive breeding: the case of rust on field pea and lentil</i> Judith Lichtenzveig	<i>Physiology of Sauvignon blanc vines, with and without Grapevine leafroll-associated virus 3, over three seasons in Marlborough, New Zealand</i> Dion Mundy
1615	<i>Characterisation of Xanthomonas spp. associated with bacterial leaf spot disease of solanaceous crops</i> Rebecca Roach	<i>Diagnosing rice grassy stunt virus in Indonesia using RT-lamp assays</i> Jane Ray	<i>Beet western yellows virus in canola crops: high infection in south-eastern Australia</i> Mohammed Aftab
1635		<i>New Tiarosporella species isolated from Australian wheat</i> Eman Barkat	<i>Measuring plant response to virus infection and water stress using quantitative composite indices</i> Roy van den Brink
1655	CLOSING PLENARY APPS awards		Sirius Room
1715	Student awards (posters/presentations) Allen Kerr Award		
1720	Invitation to forthcoming conference Acknowledgements and closing		

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The making of tree pathogens: a genomic dissection

R.C. Hamelin^A

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Tree pathogens cause some of the most devastating outbreaks in forests. In spite of their importance, we know relatively little about their origin and sources and the basic common characteristics of tree pathogens. We applied a genomics approach to understand how the poplar canker pathogen, caused by

Mycosphaerella populorum (anamorph: *Septoria musiva* syn. *Sphaerulina musiva*), evolved to cause one of the most severe diseases of poplars across various hosts and landscapes. This pathogen has recently expanded geographically and has been increased its host range to additional species such as the black cottonwood, *Populus trichocarpa* and the balsam poplar, *P. balsamifera*. Genome comparison between *M. populorum*, which causes both leaf spots and cankers, and *M. populicola*, a close relative that also attacks poplar but does not cause cankers, indicates that *M. populorum* has acquired a unique set of genes via horizontal gene transfer (HGT) that is important to its ability to attack woody hosts. Population genomic analysis of *M. populorum* samples from across its distribution range highlights an unusually high and uneven level of diversity even in recently introduced populations. The patterns suggest both extensive migration and possibly additional unsampled ghost populations that could have contributed to the recent expansions. We hypothesise that a jump to *P. balsamifera* could be driving speciation by imposing selection at the mating type locus, causing a shift from sexual to asexual reproduction, followed by isolation and drift. These results highlight very dynamic mechanisms leading to host colonisation and incipient speciation and specialisation in this tree pathogen.

Multi-function AVE1-like effectors in scab pathogens

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INTRODUCTION

Effector candidates in *Venturia* pathogens that cause scab disease of apple and pear have been identified using genomics, transcriptomics and proteomics. Most fungal effectors are genus-, species- or race-specific, however a few are more broadly conserved. A single *ECP6* ortholog is found in all *Venturia* genomes sequenced, however numerous copies of *Ave1* and *AvrLm6*, have also been identified. Single orthologs of *Ave1* from *Verticillium dahliae*, a virulence effector that activates *Ve1*-mediated resistance in tomato, have been identified in unrelated fungal phytopathogens (*Colletotrichum higginsianum*, *Cercospora beticola*, *Fusarium oxysporum* f. sp. *lycopersici*). *Ave1* also shares similarity to a single ortholog in the phytopathogenic bacterium *Xanthomonas axonopodis* pv. *citri*, as well as to a common family of plant natriuretic peptides involved in plant homeostasis.¹ We have identified a large *Ave1*-like gene family (encoding predicted proteins with conserved cysteine patterns and 37-57% overall amino acid identity) in the hemi-biotrophic apple scab fungus, *Venturia inaequalis*.

A conserved intron in the 5' UTR causes problems for automated gene calling of *Ave1* orthologs in *Venturia* (transcriptome data exacerbated, but proteomics alleviated the problem), with several *Ave1* proteins assisting ID of new *Ave1* loci². Several of the *Venturia* orthologs are up-regulated during leaf infection (RNAseq), and some are also highly expressed during *in vitro* growth on cellophane².

MATERIALS AND METHODS

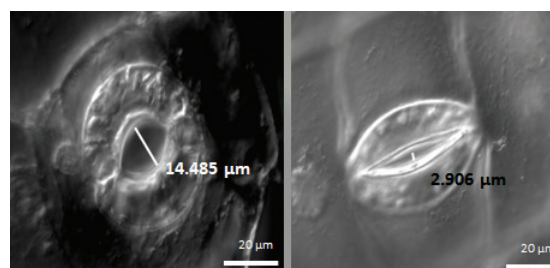
The two most highly expressed *V. inaequalis* *Ave1* orthologs, Vi936atg12189 and Vi301atg3613, are also most similar to the homeostasis regulating *Arabidopsis thaliana* protein At-PNP-A. We synthesised 39 amino acid peptides of the homeodomain (hd) of both *V. inaequalis* proteins and the corresponding 36-amino acid peptide of AtPNP-A. *Arabidopsis* mesophyll protoplasts were exposed to 0.1 μ M of each peptide for 15 mins.

We also exposed *Tradescantia fluminensis* leaves to 0.1 μ M of each peptide +/-0.1 μ M ABA for 2 hrs, stomatal apertures were then measured.

RESULTS AND DISCUSSION

We confirmed AtPNP-A causes swelling of the protoplasts³ and showed both *V. inaequalis* peptides cause protoplasts to swell (data not shown). In the absence of any peptide, ABA causes stomatal closure, and AtPNP-A peptide inhibits stomatal closure in the presence of ABA³. Stomata close in the presence of both *V. inaequalis* peptides, even in the absence of ABA (Fig 1).

A



B

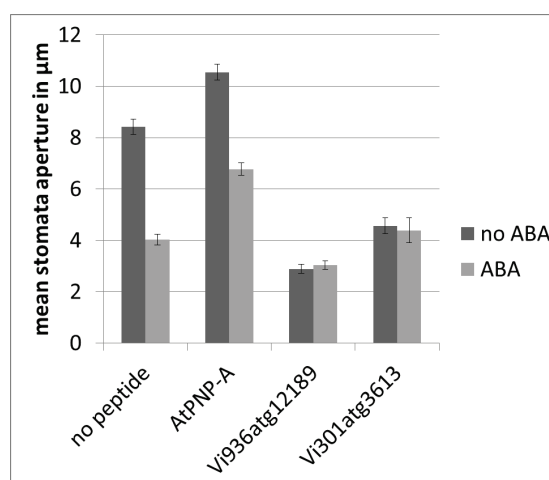


Figure 1. *Tradescantia fluminensis* stomatal aperture measurements. A, Open (left), closed ABA-treated (right). For each treatment at least 20 stomatal apertures were measured and three independent replicates were done (B). Mean aperture is shown. Error bars are SE.

We hypothesise that the *Venturia* *Ave1*-like proteins may play a role during biotrophic infection in disturbing plant homeostasis and promoting nutrient release from plant cells. Closing stomata may be beneficial for *Venturia* during biotroph, in reducing water loss due to breaches in the cuticle of infected leaves. We will also test the reaction of fungal protoplasts to these peptides.

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Comparative analyses used to investigate host-specificity determinants in *Venturia pirina* and *V. nashicola*

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INTRODUCTION

Pear scab is a major fungal disease of pears worldwide; it is caused by two distinct species, *Venturia pirina* (causes European pear scab) and *V. nashicola* (causes Asian pear scab). The two species display host specificity: *V. pirina* exclusively infects European pears (*Pyrus communis*); while *V. nashicola* exclusively infects Asian pears (*P. bretschneideri*, *P. ussuriensis* and *P. pyrifolia*)². The incompatible relationships between European pears and *V. nashicola*, and Asian pears and *V. pirina*, are considered to be governed by non-host resistance^{1,2}; however, the multi layered, molecular mechanisms of non-host incompatibility is poorly understood. The *Pyrus-Venturia* pathosystem provides a unique opportunity to dissect the underlying genetics and to understand host pathogen coevolution of this potentially more durable form of resistance. Early defences from plant hosts in response to a non-adapted pathogen are likely to be governed by recognition of conserved pathogen-associated molecular patterns (PAMPs). Recognition results in PAMP-triggered incompatibility (PTI) and the pathogen is unable to successfully infect the host. In adapted pathogens, PTI may be triggered, but is suppressed by small, secreted proteins from the pathogen, known as effectors. The distinction between the host specificity of *V. pirina* and *V. nashicola* may well be largely determined by differences in their effector arsenal³. Fluorescence phenotyping has been used to characterise responses to infection with different isolates of *V. pirina* in European, Asian and inter-specific pear accessions. Furthermore, effector candidates exclusive to *V. pirina* have been investigated to determine their role during infection.

MATERIALS AND METHODS

Fluorescence phenotyping: Asian, European & inter-specific *Pyrus* species were inoculated with European pear scab isolates for phenotypic characterisation of resistance. Cleared and stained leaf samples were examined by epifluorescence microscopy under brightfield (BF) and ultraviolet (UV) lighting conditions to describe the interactions.

Bioinformatic analysis of host-specificity determinants:

Host-specificity candidate genes were identified using a comparative secretomics. Candidates were identified in whole genome sequences of *V. nashicola* & *V. pirina* screened across a collection of isolates (isolated in Japan, Israel, China, NZ and Australia) to determine presence/absence sequence polymorphisms and evidence of selection pressure.

RESULTS

All Asian pear accessions showed resistance to *V. pirina* (no or limited interaction, or apparent hypersensitive response, HR) (Fig 1e, f). Inter-specific varieties showed a range of fluorescent phenotypes, including susceptible and possible

HR-like reactions (Fig 1h, j). European accessions were all susceptible as expected (Fig 1a, b)

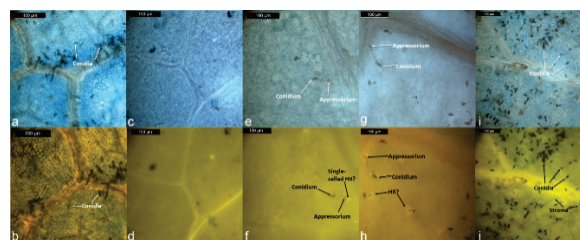


Figure 1. Micrographs of pear leaves inoculated with *Venturia pirina* isolates. a, b, Susceptible interaction on European pear c.v. Gorham; c, d control treatment (no inoculum, water only); e, f, no or limited interaction on Asian pear, Kosui; g, h, possible HR-reaction on inter-specific pear accession; i, j, susceptible interaction on inter-specific pear accession.

FUTURE DIRECTIONS

Gene expression analyses of candidates to investigate role during early infection in compatible and incompatible (host and non-host resistance) interactions

Identify genes present in genomes as singletons to avoid potential functional redundancies present in gene families. Functional analysis such as cloning host-specificity candidate gene of interest to assess role during infection

ACKNOWLEDGEMENTS

Plant Biosecurity CRC, La Trobe Post-Graduate Research Award. Thank you to Australasian Plant Pathology Society (APPS), Phytopathology Society of Japan (PSJ), and the Australia Japan Foundation for the Student Exchange to Japan Scholarship, APPS for financial support to attend the 20th APPS Conference, Fremantle, 2015.

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The secretome of *Pyrenophora teres* f. *teres*: role of virulence-associated proteins during net blotch disease of barley

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INTRODUCTION

The fungus that causes net form net blotch disease (NFNB) of barley, *Pyrenophora teres* f. *teres* (*Ptt*), spends most of its lifecycle as a necrotroph causing extensive leaf necrosis on susceptible barley varieties (1).

We have previously shown that proteins in the culture filtrates of *Ptt* can cause NFNB symptoms in a susceptible barley cultivar (2,3) suggesting that *Ptt* secretes a number of virulence-related proteins such as toxins and enzymes involved in cell wall degradation. Indeed, we recently identified an endoxylanase (PttXyn11A) that was produced to a greater extent in a more virulent *Ptt* isolate (4). However, whether the proteinaceous secretome varies among a large number of *Ptt* isolates varying in their virulence on different barley cultivars was unknown. The objective of this research was therefore to identify and characterise the role of individual proteins isolated from the secretomes of 28 *Ptt* isolates which differ in virulence on Australian barley varieties.

MATERIALS AND METHODS

Twenty eight single-spore *Ptt* isolates were chosen for protein production based on a number of parameters: the variety from which the isolates were collected, when isolates were collected, and how virulent they were on key varieties and breeding lines. Isolates of *Ptt* (provided by South Australian Research and Development Institute and Department of Agriculture Forestry and Fisheries Queensland) were grown in Fries culture medium (FCM) and toxins were extracted as per Sarpeleh *et al.* (2008) (2) using filtration. A polyacrylamide gel electrophoresis (PAGE) and two-dimensional gel electrophoresis (2DGE) proteomics approach was used to generate the secretome profiles. These profiles were then compared to allow identification of protein spots or bands associated with virulence. Unique spots or bands were selected and analysed at Adelaide Proteomics Centre (The University of Adelaide) using Liquid Chromatography-Electrospray Ionisation Ion-Trap Mass Spectrometry (LC-ESI-IT MS). Bioinformatic analysis of the peptide sequences was then used to determine the likely function of the candidate proteins.

To further confirm that the toxins were produced by the fungus *in planta*, the gene expression of the individual virulence candidates was also monitored during the interaction between a susceptible barley variety and a more virulent *Ptt* isolate. Total RNA was extracted from infected plants during the interaction at 24, 48, 96, 144 and 168 hours post inoculation) and cDNA synthesised and used in qPCR to evaluate the gene expression profiles during the interaction. The gene expression profiles were then used to choose a subset of virulence-associated proteins to study in further detail (via heterologous expression).

RESULTS AND DISCUSSION

A total of 260 proteins were identified as being uniquely produced by isolates of *Ptt* with specific virulence on barley, especially post-2009 (such as virulence on the varieties Keel or Maritime) with 50 of these proteins being common to extremely virulent isolates. Bioinformatic analysis has

enabled the proteins to be categorised according to potential function. The majority of proteins appear to have no known function but a number share similarity with those that have been identified as important in other plant-pathogen interactions. These include having roles in fungal adhesion and growth, cell wall degradation, necrosis induction or suppression of plant defence responses. The unknown proteins were also analysed for motifs and cysteine-richness considered common to effectors (which enable host-specificity of the response) allowing the identification of several candidates. The gene expression of all 260 proteins was monitored using qPCR during the plant-pathogen interaction to show that each was produced *in planta* and the timing of their production in relation to symptom development during NFNB was established. Candidates were then chosen based on their likely role, protein size, cysteine content, pattern of expression during the interaction and the extent of that expression. Although most of these have unknown function, based on the bioinformatics analysis the candidates include ceratoplatenin, cupin-like protein, bifunctional glucose/aldose epimerase, isochorismatase, endo- and exo-glucanases, glycosylphosphatidylinositol (GPI)-anchored common in fungal extracellular membrane (CFEM) domain-containing protein, ABC-transporter protein, spherulin 1a precursor, cutinase, and a leucine rich repeat (LRR) protein. The information that we have gathered from this secretome analysis has provided some insight to how various fungal proteins affect the interaction between *Pyrenophora teres* f. *teres* and barley. We will present a model of the role these proteins play in relation to symptom development in NFNB disease of barley.

ACKNOWLEDGEMENTS

This work is funded by the Grains Research and Development Corporation. Thanks to Shubhobrotto Das for technical assistance.

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Concurrent session 1a—BIOLOGY: plant pathogen

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21

Transcription regulators of aphid resistance in *Medicago truncatula*

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INTRODUCTION

Aphids are sap-sucking insects which cause serious damage to agriculture by depleting plant nutrients and vectoring pathogenic viruses. Resistance to aphids has been identified in a number of plant species; however the molecular mechanisms underlying aphid resistance remain largely unknown.

The legume *Medicago truncatula* has emerged in recent years as an effective model to study *R* gene-mediated aphid resistance. A single *M. truncatula* line, Jester, has been shown to contain three independent *R* genes that confer strong resistance to three different aphid species (1).

Transcription factors (TFs) have been shown to be key regulators of the plant's defence response. Our previous study suggested that TFs are among the early regulatory genes in resistant plants against the infestation by bluegreen aphid and may play an important role in resistance to multiple aphid species (2).

The transcription profiling of over 1000 *M. truncatula* TF genes in this study led to the identification of TFs which are potentially involved in the resistance against three aphid species.

MATERIALS AND METHODS

Plants Included in this study were the *M. truncatula* reference genotype A17 (aphid susceptible) and its closely-related line Jester which contains three single dominant genes conferring resistance to three aphid species (3).

Aphid infestation The three aphid species studied were: bluegreen aphid (BGA), pea aphid (PA) and spotted alfalfa aphid (SAA). Twenty adult aphids were used to infest a single trifoliolate leaf of A17 or Jester. The leaf samples were collected at 6 and 12 hours after aphid infestation.

TF profiling Over 1000 *M. truncatula* TFs were analysed using high-throughput quantitative real-time PCR technology following the procedures as described previously (2).

RESULTS

Over 100 TF genes showed differential expression (up or down-regulation) in the susceptible and resistant plants upon the infestation by the three aphid species.

There was minimal overlap of the differentially expressed genes in response to the three aphid species.

However, members of the *WRKY* gene family were induced by all three aphid species. The transcription levels were higher in the resistant interaction than the susceptible interaction and closely associated with the aphid resistance gene (Figure 1).

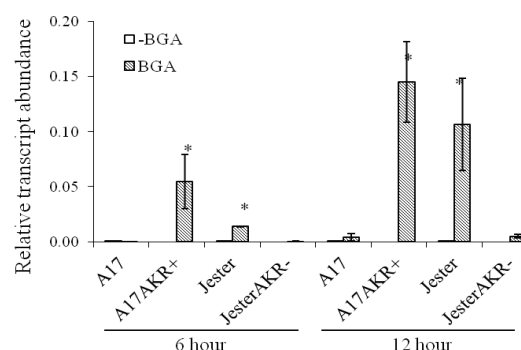


Figure 1. Relative transcript level of a WRKY transcription factor gene in the *M. truncatula* susceptible (A17 and JesterAKR-), resistant (Jester and A17AKR+) plants following the infestation by BGA for 6 and 12 hours.

DISCUSSION

The induction of the WRKY gene members in the *M. truncatula* resistant Jester by all three aphid species suggested they play a key role in resistance to aphids. Further study using the knock-out mutants and over-expression of these WRKY members in relation to aphid performance should help to justify this hypothesis.

ACKNOWLEDGEMENTS

We thank Elaine Smith for technical support, a CSIRO Transformational Biology-Acorn Grant to fund the TF profiling work and The Samuel Roberts Noble Foundation to facilitate this project.

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High coverage *in planta* RNA sequencing identifies *Fusarium oxysporum* effectors and *Medicago truncatula* resistance mechanisms

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INTRODUCTION

Fusarium wilt disease caused by the root-infecting fungal pathogen *Fusarium oxysporum* is a major constraint to global grain legume production including chickpea, lentils and lupins, and in Australia represents a significant biosecurity threat to these industries. Globally there is no effective curative treatment, with the use of resistant cultivars the main control mechanism. However, new races can develop and overcome the static nature of *R*-genes. Thus, understanding the nature of pathogenicity in *F. oxysporum* is central to its control. To gain molecular insight into the *F. oxysporum*-legume interaction, we developed a model pathosystem using the model legume *Medicago truncatula*. Through high coverage RNA sequencing (RNAseq) of infected *Medicago* roots we have successfully identified both novel *F. oxysporum* pathogenicity and effector genes, and unravelled possible mechanisms of host resistance.

MATERIALS AND METHODS

Disease assays The pathosystem was developed as described in (1) using *M. truncatula* and the causal agent of *Fusarium* wilt on *Medicago* species *F. oxysporum* forma specialis *medicaginis* (*Fom*-5190a/*BRIP* 5190 a).

RNA sequencing RNA isolation and sequencing are described in (1). Briefly, RNA was extracted from *Fom*-5190a grown *in culture* and from infected *Medicago* roots 2 days post inoculation (dpi). Stranded Illumina TruSeq libraries were sequenced (100bp PE reads) on an Illumina HiSeq platform. 51-60 million reads were generated for each sample. *Fom*-5190a and *Medicago* reads were mapped to the *Fom*-5190a or *Mt4.0v1* reference genomes respectively, followed by differential expression analysis.

RESULTS AND DISCUSSION

Strong host resistance identified Screening of 100 core *Medicago* accessions identified strong resistance with a major resistance loci mapped (*Resistance to Fusarium oxysporum*1, *RFO1*). Susceptible plants are extensively colonised, with increased fungal proliferation within roots (Fig. 1).

RNAseq guided identification of *Medicago* resistance mechanisms To identify *RFO1* candidates and differential defense responses in resistant vs susceptible plants, we compared their root transcriptomes following *Fom*-5190a inoculation. Over 1500 genes were significantly up-regulated >2-fold in the resistant line. Gene Ontology (GO) term analysis identified significant enrichment of defense and signalling processes in the resistant plants including several transcription factors (Table 1). Manipulation of these transcription factors may enable broad spectrum resistance/tolerance. Towards cloning *RFO1*, analysis of *RFO1* loci gene expression and association-type mapping data from diverse *Medicago* accessions identified 6 strong *RFO1* candidates.

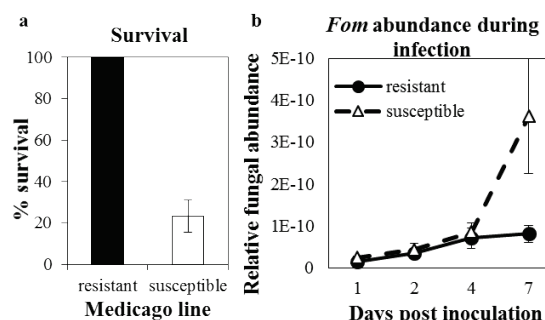


Figure 1. (a) Survival of resistant versus susceptible *M. truncatula* 21 dpi. (b) Relative *Fom*-1590a (*Fo18s*) abundance in root tissues. Samples are averages $\pm$ SE.

Table 1. Biological processes up-regulated in resistant over susceptible plant roots at 2 dpi. (FDR $p \leq 0.05$).

GO Term	Description	p-value
GO:0006952	defense response	1.80E-43
GO:0006950	response to stress	3.30E-38
GO:0050896	response to stimulus	3.80E-37
GO:0007165	signal transduction	3.00E-12
GO:0023046	signaling process	3.00E-10

RNAseq guided identification of *Fom*-5190a effectors

Based on fungal effectors characterised as small, secreted proteins (SSPs), we predicted 576 in *Fom*-5190a with 71 predicted to reside on conditionally dispensable chromosomes (CDCs) known for their roles in pathogenicity (2). Using up-regulation during infection as an indicator of effector function, we identified a high confidence effector set with most located on CDCs. The list includes novel effectors as well as those with differing degrees of similarity to other hypothetical *F. oxysporum* or plant pathogenic fungal proteins, including several characterised *F. oxysporum* 'Secreted In Xylem' effectors.

ACKNOWLEDGEMENTS

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Persistence of fungicide efficacy on mandarin fruit

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INTRODUCTION

Subtropical citrus production is hampered by 'Emperor' brown spot (EBS) disease caused by *Alternaria alternata*. EBS symptoms include lesions on fruit and young leaves, as well as blighting of young shoots. Managing EBS relies on protective applications of fungicides.

Ensuring that fungicide use is efficient and effective requires a thorough understanding of the persistence of efficacy over time. Presently, fungicides are applied at an arbitrary 4-5 week interval with little regard for differences between the fungicides.

This study aimed to compare the persistence of fungicide efficacy using *A. alternata* as a bioassay (1).

MATERIALS AND METHODS

Field application of fungicides Fungicides were applied to four replicate trees of the 'IrM2 Murcott' (*Citrus × aurantium*) in a randomised complete block design within a commercial orchard at Mundubbera, Qld. The fungicides were applied when the fruit were ~45 mm diameter using a hand lance sprayer. Four trees that did not receive fungicide were included as a control. The fungicides included mancozeb, azoxystrobin and iprodione, as well as four experimental fungicides, two multisite activity fungicides and two succinate dehydrogenase inhibitor (SDHI) fungicides.

Bioassay Ten fruit were collected from each data tree for bioassay at 1, 11, 21, 33 and 64 days after application of the fungicide. Each fruit was inoculated with conidia of *A. alternata*. Fruit were incubated at high humidity in sealed plastic containers for 72 hours. After incubation disease severity was rated as: 0 = no lesions; 1 = 1 to 5 lesions; 2 = >5 lesions. The percent disease control relative to the untreated control was determined for each time point, and the data analysed by ANOVA.

RESULTS

Significant differences in disease control were observed between the fungicides over time, with the major decline in efficacy observed between days 1 and 11 (Fig. 1). Beyond 21 days there was no further significant decline in efficacy. Iprodione (Fig. 1a) and multisite A (Fig. 1b) had the highest efficacy at days 1 and 11. Several of the other fungicides showed equivalent efficacy to multisite A at day 1, but these fungicides fell to their lowest efficacy by day 11. SDHI B and azoxystrobin showed no significant changes in efficacy throughout the bioassay.

DISCUSSION

Comparison of the persistence in efficacy against *A. alternata* of several fungicides has shown the potential for rapid declines in fungicide efficacy within as little as 10 days after application. Rankings of efficacy decline loosely correlate with observed efficacy of the fungicides in the field (2, unpublished data). However, it is expected that the bioassay favours fungicides which inhibit spore germination, over those inhibiting mycelial growth. A number of factors influence the persistence of fungicides in the field such as rainfall (1) and fruit expansion (3). Our data

shows that fungicides respond differently to these influential factors, and this should be considered when determining fungicide application intervals.

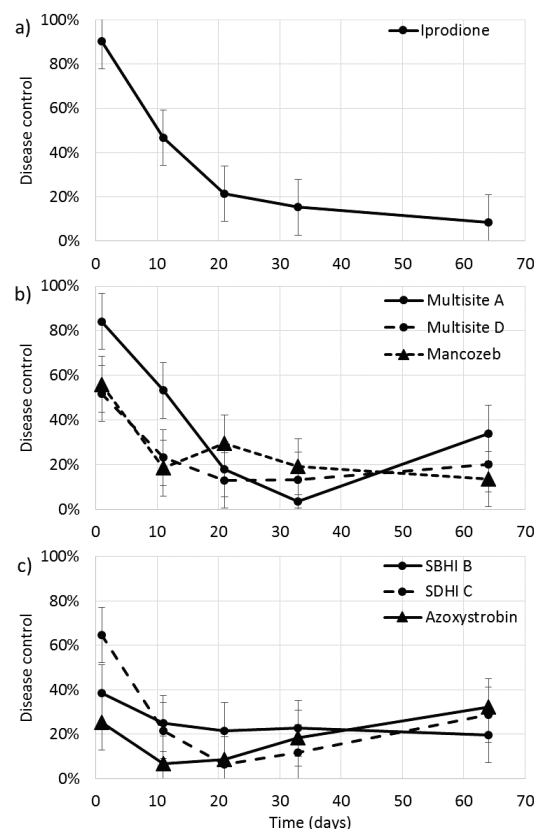


Figure 1. The persistence of efficacy of several fungicides against *A. alternata* on detached 'IrM2' fruit. Bars represent the least significant difference.

ACKNOWLEDGEMENTS

We thank Horticulture Innovation Australia Ltd & Craig Wallis (Auburnvale Citrus) for supporting this study.

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Foliar phosphite application has minor phytotoxic impacts across a diverse range of conifers and woody angiosperms

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INTRODUCTION

Phytophthora plant pathogens cause tremendous damage in planted and natural systems worldwide. Phosphite is the only effective pesticide, to control broad scale phytophthora disease; however, limited knowledge of the phytotoxic and physiological effects of phosphite on a broad range of endemic New Zealand plants is a barrier to disease management and incursion responses (1).

MATERIALS AND METHODS

The phytotoxic impact of phosphite, applied as foliar spray at 0, 12, 24 and 48 kg a.i. ha⁻¹ on thirteen conifer and angiosperm species native to New Zealand, and two exotic coniferous species was assessed. Phytotoxic impacts were determined by digitally quantifying the area of necrotic tissue (2), and measuring chlorophyll-a-fluorescence parameters (optimal quantum yield, Fv/Fm; effective quantum yield of photosystem II, ΦPSII). In addition, stomatal conductance (gs) was measured on a subset of six species.

RESULTS

Significant necrosis occurred in only three species: in the lauraceous canopy tree *Beilschmiedia tawa* (8-14%) and the understory shrub *Dodonaea viscosa* (5-7%) across phosphite concentrations and solely at the highest concentration in the myrtaceous pioneer shrub *Leptospermum scoparium* (66%), (Figure 1). The parameters Fv/Fm, ΦPSII and gs remained unaffected by the phosphite treatment (Data not presented here).

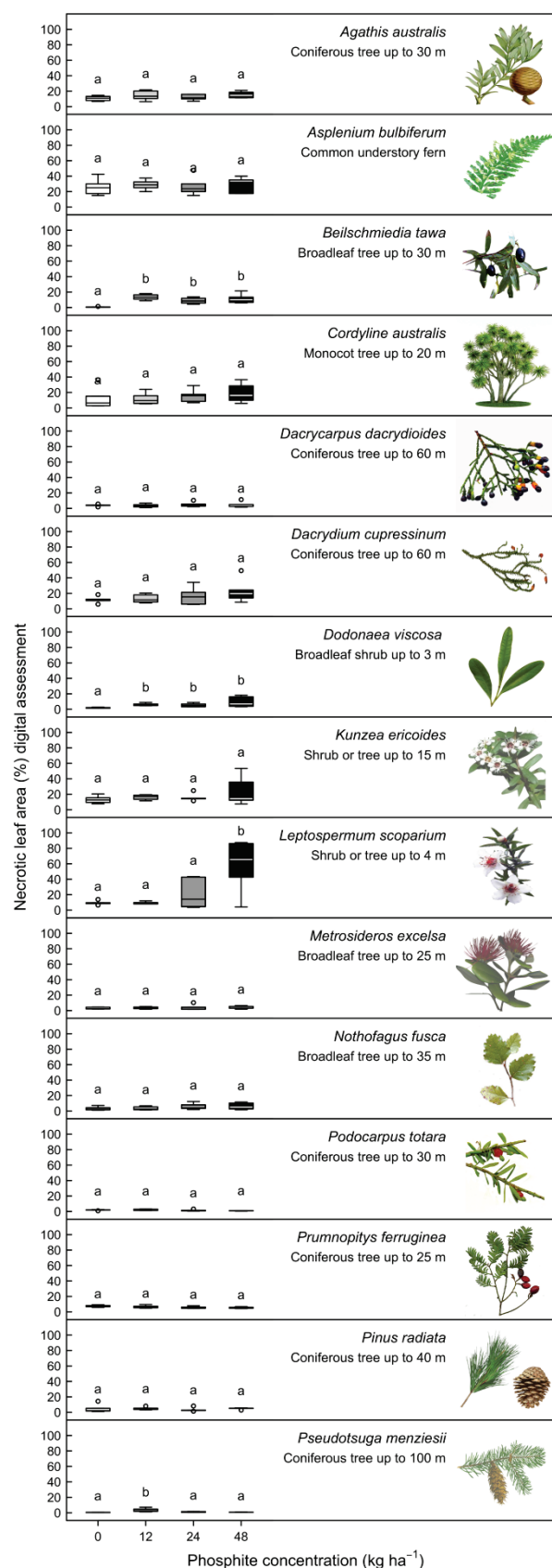
DISCUSSION

Our findings suggest minimal phytotoxic effects resulting from foliar phosphite application across diverse taxa and regardless of concentration. Although long-term studies are required to ascertain potential ecological impacts of repeated phosphite applications, our study supports the use of phosphite as a management tool to control plant diseases caused by phytophthora pathogens in plantations and natural ecosystems.

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Figure 1 (adjacent). Proportion of necrotic leaf area that was digitally assessed using WinFOLIA of 13 native keystone species and two important New Zealand forestry species as a function of foliar phosphite application (white = control, light grey = 12 kg ha⁻¹, grey = 24 kg ha⁻¹, black = 48 kg ha⁻¹). Different lower case letters indicate statistically significant differences at $\alpha = 0.05$ (multiple comparison procedure using Tukey contrasts).



Resistance of *Erysiphe necator* to QoIs and DMIs

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INTRODUCTION

Powdery mildew, caused by the obligate biotrophic fungus *Erysiphe necator*, is the most economically important disease of grapevines in Australia. Disease management is dependent on the timely application of fungicides, which include the Quinone outside Inhibitors (QoIs) and Demethylation Inhibitors (DMIs). Resistance to the QoIs in grapevine powdery mildew is conferred by the G143A mutation, a single point mutation in the *cytb* gene (1). The Y136F mutation, in the *cyp51* gene, is associated with the development of resistance to the DMIs, however, other mechanisms are also thought to contribute (1). Whilst resistance to QoIs (2) and DMIs (3) has been found within Australia, the overall extent of resistance is not clear. Here, we aim to determine the extent of resistance to the QoI pyraclostrobin and the DMI penconazole in populations of grapevine powdery mildew obtained from Australian vineyards.

MATERIALS AND METHODS

Forty seven isolates were obtained from powdery mildew infected leaves or bunches in 2013/14. Cultures were maintained as single spore isolates on surface sterilised detached grapevine leaves, cv. Cabernet sauvignon, on water agar. Isolates were tested in leaf-disc assays for sensitivity to pyraclostrobin (Cabrio®) and penconazole (Topas®), using concentrations from 0.001 to 10 µg/mL active ingredient. The EC₅₀ µg/mL, the dose that reduces growth by 50%, was calculated by Probit analysis (Genstat 16th ed). Detection of the G143A and Y136F mutations was carried out by extraction of genomic DNA followed by Sanger sequencing of the *cytb* and *cyp51* genes.

RESULTS AND DISCUSSION

Pyraclostrobin: The EC₅₀ values were between 0.0005 to 14.4 µg/mL with the overall mean 1.6 +/- 3.0 (sd) (n=47). The G143A mutation was detected in all isolates with an EC₅₀ value > 0.11 µg/mL (Fig 1). This confirms the link between reduced sensitivity to pyraclostrobin and the presence of the mutation. The bimodal distribution pattern of the wild type versus mutant isolates shows a definite sensitivity shift towards resistance (Fig 1). This pattern is consistent with major single gene resistance described for the QoIs (1). The G143A mutation was detected in 68% of isolates genotyped to date (n=37). Testing is continuing, but even with the limited numbers there is good correlation between exposure to strobilurins and presence of the mutant (data not shown).

Penconazole: The EC₅₀ values ranged from 0.0004 to 1.3 µg/mL with the overall mean 0.2 +/- 0.2 (sd) (n=45). The lack of obvious pattern of the wild type versus mutant genotypes (Fig 2) may be due to the small number genotyped to date (n=20), with the Y136F mutation detected in 55% of samples. Unlike the strobilurins, the correlation between exposure and presence of mutant is not as well defined (data not shown). A relationship may be clearer following further genotyping, however although the Y136F mutation is associated with DMI resistance, it is possible that there are other mutations as well as an accumulation of other resistance mechanisms (1).

Overall, these results have implications for resistance management strategies in the viticultural industry. This high proportion of mutants detected is of major concern, indicating that resistant populations are common and without effective management the potential for loss of field efficacy is significant.

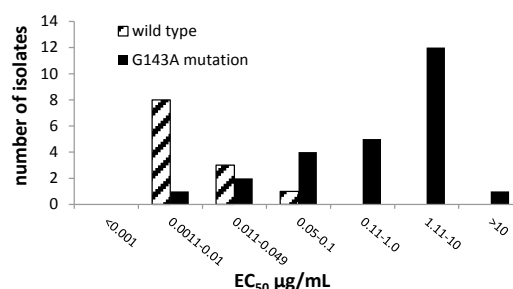


Figure 1. The distribution of sensitivities and genotype frequency of isolates of *Erysiphe necator* tested with Cabrio®.

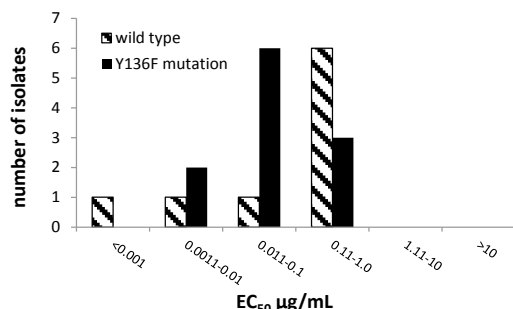


Figure 2. The distribution of sensitivities and genotype frequency of isolates of *Erysiphe necator* tested with Topas®.

ACKNOWLEDGEMENTS

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Efficacy of soil amendment to control verticillium wilt disease

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INTRODUCTION

Verticillium dahliae can survive in the soil for many years in the form of microsclerotia (MS). These microsclerotia are responsible for establishing new infection of potato plants. Reduction in soil inoculum has always been the interest of disease control strategies. Besides potato, eggplant is also susceptible to *V. dahliae*. Eggplant is more amenable to study yield loss assessment in pot trials and can be used as a model to study the efficacy of soil amendment to suppress verticillium wilt disease. The aim of this study was to assess the efficacy of soil amendments to reduce verticillium wilt disease.

A. Soil microcosm assay. Microcosm assays were established to study the suppression of MS germination after MS were incubated in field soil supplemented with sulphur and brown coal soil amendments. Both 1% sulphur and brown coal reduced the germination of MS in two trials as high as 94% and 86% after 4 weeks incubation respectively, while for 10% sulphur and brown coal the germination was reduced by 100% and 75% respectively.

B. Inoculum optimisation. Both potato cv. Russet Burbank and eggplant cv. Black Beauty seedlings were planted into seedling mix containing three different inoculum (MS) concentrations. Eggplant seedlings were also planted into field soil with the same treatments. Severity of foliar symptoms was determined using 0-5 visual qualitative scale (1). Infected potato plants showed moderate to severe disease severity at 500 MS CFU/g soil, while low to moderate in infected eggplants. Thus, this concentration was used in amendment glasshouse trials.

C. Soil amendment glasshouse trial. Eggplant seedlings were planted in field soil that was supplemented with amendment and MS inoculum. Severity of foliar symptoms was determined using 0-5 visual qualitative scale (1). Plant height and biomass dry weight were measured at the end of the trial. The efficacy of sulphur and brown coal to reduce verticillium wilt disease was partly successful with a decrease in incidence of infection in each treatment. However, severity of infection was the same as the MS control and sulphur had severe phytotoxic effect to the plants. Sulphur reduced eggplant height and yield in comparison to the control which also found in previous study (2). Further glasshouse trials are on the way to confirm above findings.

CONCLUSION

1% Sulphur and brown coal inhibited MS germination in microcosm assays. Verticillium wilt was successfully established in potato and eggplants in pot trials using 500 MS CFU/g soil. Soil amendments reduced the incidence of infection in pot trials with eggplants.

ACKNOWLEDGMENT

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Phosphite for control of kauri dieback

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INTRODUCTION

Kauri dieback, caused by *Phytophthora agathidicida* (4), also known as *Phytophthora* taxon Agathis (PTA), is a serious problem in some kauri (*Agathis australis*) forests in Northern New Zealand (1).

Phosphite (phosphorous acid) provided good protection from *P. agathidicida* in glasshouse trials with inoculated kauri seedlings (2). The current work investigates the efficacy of phosphite in treating infected forest trees. Results 3 years into a 5-year programme are reported. Aspects of this work are discussed in more detail elsewhere (3).

MATERIALS AND METHODS

Site selection and treatment. Four kauri forest sites were selected for trials: Raetea and Omahuta Forest in the Mangamuka ranges, Northland, and Huia and Whatipu in the Waitakere ranges, Auckland. All four sites had confirmed diagnoses of *P. agathidicida* presence, and all 162 trees selected for the trial showed symptoms of kauri dieback (canopy thinning, dieback and/or bleeding lesions on the lower trunk). All lesions were measured, lesion margins were marked, and tree canopies were photographed. Treatments were randomly assigned to trees, ensuring even distribution among initial disease severity rating classes. In January to March 2012, trees were injected with phosphite (Agrifos® 600), at concentrations of 20% or 7.5%, with 20 ml of solution injected for every 20 cm trunk circumference. Untreated controls were left for comparison. One year later, half of the trees initially treated were re-treated with 7.5% phosphite.

Assessments At 6-monthly intervals, canopy health was compared to that in original photographs and trunk lesion activity and spread was noted.

RESULTS AND DISCUSSION

Lesion activity and spread was significantly greater in untreated control trees than in phosphite-injected trees. After 3 years, less than 1% of lesions in phosphite-injected trees remained active, compared with 58.5% of lesions remaining active in untreated control trees. In treated trees almost all lesions had ceased spreading and had cracked and healed around the margins, whereas in untreated control trees a majority of lesions had continued to advance. With respect to lesion activity or advance, there was no apparent difference between the high or low rates of phosphite, or a single or double application.

At the two Auckland sites, phytotoxicity symptoms including leaf yellowing, leaf loss and premature twig abscission was noted in many phosphite-injected trees within a few months of treatment. Occasional bark cracking in line with injection points was also noted. At these sites, 15% of phosphite-injected trees died within 3 years, in most cases following injection with the high (20%) rate of phosphite. In all cases, the trees that died were showing severe kauri dieback symptoms at the start of the trial. In the two Northland sites, only minor phosphite toxicity symptoms were noted and no injected trees have died. At

Raetea, four out of 14 control trees have died, in all cases following rapid *Phytophthora*-lesion spread and trunk girdling.

Table 1. Lesion activity and expansion in *P. agathidicida*-infected kauri trees treated with high (20%) or low (7.5%) rates phosphite or left untreated. Letters indicate significance groupings ($P < 0.01$). Treatments applied January 2012/January 2013. Assessments were made 3 years after initial treatment.

Treatment	Lesions active (%)		Mean Lesion expansion (cm)	
Control	58.5	a	12.65	a
High/Low	0	b	0.45	b
High/nil	0	b	0.10	b
Low/Low	1.9	b	0.64	b
Low/nil	0	b	0.33	b

There is good evidence to date that trunk injection with phosphite reduces the spread of *P. agathidicida* lesions in kauri trees. Following treatment, lesions stopped growing and healed. Thus, phosphite is a potential treatment for kauri dieback. However, phytotoxicity symptoms noted on some trees following injection, particularly with the 20% rate but also to a lesser extent with the lower rate, mean that more work is required to find a safe balance between doses adequate to suppress *P. agathidicida* yet are not toxic to kauri.

ACKNOWLEDGEMENTS

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Post-harvest treatment of taro corms to minimise biosecurity risks from plant-parasitic nematodes

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INTRODUCTION

Taro (*Colocasia esculenta*) is an important export crop for the Fijian economy and a source of income for people living in rural areas. Currently Fiji exports ~ 10 000 tonnes of taro annually to markets in Australia, New Zealand, and USA. Most of the taro is produced on small to medium sized farms and is sourced by suppliers who clean and pack the produce for export.

Soil dwelling and plant-parasitic nematodes (PPN) are often intercepted with fresh taro exports and can result in: mandatory fumigation; reshipment; or destruction of the consignment (1). Fumigation increases the costs and reduces the shelf life of the produce. The detection of nematodes in a taro consignment also increases the biosecurity clearance times which further reduces the shelf life and causes delays or disruptions in the supply chain.

In this study we evaluate the effectiveness of a commonly used postharvest treatment to minimise the biosecurity risks from nematodes on fresh taro.

MATERIALS AND METHODS

Fresh taro corms destined for export were sourced at a commercial cleaning and packaging house and the postharvest treatment experiments were conducted onsite. The corms were cleaned by hand, removing root, petiole, periderm (dark brown) layer and any propagative buds. The corms were washed in tap water to remove any soil on surface and placed in plastic baskets ready for packing (currently used treatment). **Dip treatment:** The cleaned corms were transferred to a 500 L plastic tub with 400 L of water mixed with 840 mL of 10% Sodium hypochlorite (NaOCl) to achieve 200 ppm available chlorine concentration (pH ~ 6-7). The corms were dipped in the solution for 5, 10, 15, 20, 25 and 30 minutes. The pH and available chlorine concentrations were monitored throughout the experiment. Random samples (N=100) were taken pre dip treatment and post dip treatment from the cortex layer of the corms and observed for the presence of nematodes. The number and type of nematodes recovered were recorded and data analysed using one way ANOVA.

Effect of Sodium hypochlorite on taro: Taro samples pre dip treatment (N=10) and post treatment (N= 10) were stored at 10 °C for one week (similar to storage conditions for export) and evaluated for appearance, taste and aroma (2). **Effect of Sodium hypochlorite on nematodes (In-vitro):** Living PPN extracted from fresh taro corms were placed in glass cavity blocks containing NaOCl solution (200 ppm) and the effect on nematodes was recorded.

RESULTS

The number of nematodes recovered from taro corms subjected to NaOCl dip treatment was significantly lower than untreated corms. There was no significant difference in the average number of nematodes recovered after 5 and 10 minutes dip treatments (Table 1). After cooking, there was no difference in taste and aroma of treated and untreated taro. A slight bleaching effect was noted in the corms dipped for 15, 20, 25 and 30 minutes. There was no

apparent difference in color of untreated corms and those dipped for 5 and 10 minutes.

Table 1. Effect of Sodium hypochlorite dip treatment on number of nematodes recovered from taro corms

Exposure time (min)	Number of nematodes (mean ±SE)
pre treatment	0.11 ± 0.04
5	0.04 ± 0.02
10	0.02 ± 0.01
15	0
20	0
25	0
30	0

The nematodes extracted included free living (37%), plant parasitic genera (34%), and bacterial feeding (29%). PPN exposed to NaOCl in-vitro, died after 1 to 17 minutes. Juveniles died within 1-2 minutes and were not recognisable after 5 minutes while vermiform adults died after 10-17 minutes.

DISCUSSION

The removal of periderm layer of taro corms followed by washing and dipping in NaOCl (200ppm) for 5 minutes, is an effective method for disinfecting ecto-parasitic nematodes associated with taro corms. Removing the taro periderm layer partly removes ecto-parasitic nematodes. Some nematodes still remain attached with soil sticking to the corms in small crevices therefore washing the corms after cleaning further reduces the numbers of nematodes. The removal of soil and surface debris, increases the efficacy of the subsequent NaOCl dip treatment. NaOCl is a strong oxidising agent, acts on nematodes present on the surface, rupturing their cuticle and causing death. The effect on endo-parasitic nematodes is yet to be studied however majority of the nematodes associated were ecto-parasitic or juvenile stages of endo-parasitic genera. NaOCl dip effectively reduces the number of nematodes and is widely used on fresh fruits and vegetables postharvest, to reduce the number of other microorganisms e.g. bacteria and fungi, reducing rot thus extending shelf life of produce (3). It is cost effective and can be adopted by taro packaging house to minimise biosecurity risks.

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Transcriptome of the foliar gymnosperm pathogen *Dothistroma septosporum*

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INTRODUCTION

Next-generation sequencing has revolutionised the study of plant-pathogen interactions, but very few such studies have been done with gymnosperm pathogens such as those infecting conifers. The pine needle pathogen *Dothistroma septosporum* causes dothistroma needle blight (DNB), a disease of global importance to both natural and planted forests, and its genome has been sequenced (1). To better understand how this pathogen interacts with its host we sequenced mRNA from three stages of the disease cycle. The transcriptome provides insights into hemi-biotrophic lifestyle transitions of *D. septosporum* and is a useful resource for guiding functional studies of virulence factors in disease.

MATERIALS AND METHODS

Clones of *Pinus radiata* were inoculated with spores of *D. septosporum* and incubated under high moisture conditions as described previously (2). Samples were taken at three time points based on the disease phenotype: 'early' (germinating spores, epiphytic growth and penetration through stomatal pores), 'mid' (mesophyll colonisation and initial lesion formation) and 'late' (mature sporulating lesions). RNA samples were extracted for each stage (with biological duplicates). RNA was also extracted from culture-grown *D. septosporum* for comparison (FM; Fungal Mycelium). cDNAs from these samples were sequenced by Illumina Hi-Seq. Reads were mapped to coding sequences from the *D. septosporum* gene catalogue (<http://genome.jgi-psf.org/Dotse1/>) and statistical significance of differences in gene expression determined using DEGseq (3).

RESULTS AND DISCUSSION

In total >3m *in planta* reads were mapped to *D. septosporum* coding sequences and the percentages of fungal reads in the total fungal and plant mix were estimated (Table 1). The increase in fungal reads from 0.5% at mid stage to 17.1% at late stage (Table 1) is consistent with the rapid increase of fungal biomass that occurs during this period (4).

Table 1. *Dothistroma septosporum* transcriptome statistics.

	Total reads (plant and fungal)	Mapped fungal reads ^a	% of total reads ^b
Early	683,221,605	512,742	0.1%
Mid	1,033,815,851	871,668	0.5%
Late	17,146,358	1,883,653	17.1%
Total	1,734,183,814	3,268,063	

^a Mapped to *D. septosporum* coding sequences

^b The % of *D. septosporum* reads in the total (trimmed) reads was estimated using a correction factor (fungal reads/0.7015) based on a mean of 70.15% of mapped fungal reads in the *in vitro* samples.

Analysis of selected categories of genes generally considered to have some involvement in pathogenicity was carried out, to look at numbers of genes significantly up-regulated *in planta* compared to in culture. We found enrichment in the necrotrophic stages (FM-mid and FM-late) for oxidoreductase, membrane-transporter and secondary metabolism-related genes, as well as effector

candidates (SSCPs) (Table 2). In contrast a bi-modal type pattern of enrichment for increased expression in both early and late stages was seen for predicted secreted proteins and CAZys, suggestive of distinct phases of carbohydrate metabolism.

The dataset highlighted many potential virulence and avirulence factors for future functional studies. It will be a useful resource for developing tools to screen for dothistroma needle blight in pines, as well as a model system for other foliar gymnosperm pathogens.

Table 2. Numbers of *D. septosporum* genes in selected groups significantly up-regulated in planta compared to in culture

Predicted protein type ^a	FM-Early ^b	FM-Mid	FM-Late
oxidoreductase	88 (15.1%)	195 (30.7%)	258 (20.6%)
membrane transporter	6 (1.0%)	14 (2.2%)	29 (2.3%)
secondary metabolism	4 (0.7%)	29 (4.6%)	25 (2.0%)
SSCP	6 (1.0%)	10 (1.6%)	22 (1.8%)
secreted proteins	112 (19.2%)	94 (14.8%)	222 (17.7%)
CAZy	35 (6.0%)	30 (4.7%)	79 (6.3%)
Total genes up- regulated <i>in planta</i> ^c	583	635	1253
Total DE genes ^c	1357	1592	2814

^a Secreted protein, CAZy (carbohydrate active enzyme) and SSCP (short secreted cysteine-rich protein) genes are as defined previously (de Wit et al 2012). Others based on GO terms: oxidoreductase GO:0055114; membrane transporter GO:0022857; secondary metabolism GO:0044550.

^b Genes significantly up-regulated in planta (Early, Mid or Late) compared to in culture (FM). Percentages of total up-regulated genes are shown in parentheses.

^c Only genes identified as having significant differential expression (DE) as determined by DEGseq DE analysis and a minimum of 2-fold up- or down-regulated were considered.

ACKNOWLEDGEMENTS

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Genetic variation among Australian isolates of *Fusarium oxysporum* from strawberry

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INTRODUCTION

Fusarium wilt of strawberry is caused by *Fusarium oxysporum* f. sp. *fragariae* (Fof) and is a major soilborne disease of the cultivated strawberry (*Fragaria × ananassa*) worldwide. Since the phase-out of methyl bromide and no commercially viable equivalent on the market (Hutton and Gomez, 2010), soil-borne diseases have recently caused major losses in Australia.

Disease outbreaks in Western Australia (Phillips and Golzar, 2008) and southeast Queensland (Hutton and Gomez, 2010) in the past few years have demonstrated the need for resistant cultivars. Knowledge of the diversity of the pathogen is critical to ensure that potential new cultivars are tested against a wide range of pathotypes. However, information on the genetic variation of *Fof* across Australia is unknown. An assessment of the virulence of a broad range of Australian isolates and their pathogenicity would allow the identification of highly virulent or diverse strains. These can be used to inoculate breeding lines.

The objectives of this study were to evaluate pathogenicity and variation amongst *F. oxysporum* isolates from Australia by means of pathogenicity testing, VCGs, and partial sequencing of the EF-1 α and the mtSSU gene regions.

MATERIALS AND METHODS

Fungal isolates. Isolates of *F. oxysporum* were obtained from the crowns of symptomatic plants collected from Australia.

Pathogenicity tests. In a glasshouse trial, a sample of nine isolates were used in root dip inoculations (1×10^6 conidia/mL) with the susceptible cultivar Kabarla (Hutton and Gomez, 2006). Disease assessment was taken 8 weeks post-inoculation. Isolates were classified as pathogenic based on a disease severity index, from 0 = healthy plant to 10 = plant dead.

Vegetative compatibility grouping of isolates. Nitrate utilisation mutants, were generated from 25 isolates when cultured on MM containing potassium chlorate. Combinations of nit mutants were paired to verify VCGs, determined by the isolate's ability to form a heterokaryon. If an isolate formed no heterokaryon with any other isolate it was termed a 'single member'.

PCR and partial sequencing of EF-1 α and mtSSU. Isolates were characterised using analyses of translation elongation factor 1 alpha (EF-1 α) and mitochondrial small subunit (mtSSU) ribosomal DNA. PCR products were sequenced in both directions using primers EF-3 and EF-22T primers for EF-1 α and MS1a and MS2a primers for mtSSU.

RESULTS

Pathogenicity tests Disease development varied amongst isolates. Of the nine isolates tested, five were designated as *Fof*. Pathogenic isolates underwent further tests to confirm pathogenicity.

Vegetative compatibility grouping of isolates. Six isolates were considered as incompatible. Four isolates from

Queensland grouped together (VCGa), and four isolates from Western Australia (VCGb). The remaining isolates were considered single-members.

PCR and partial sequencing of EF-1 α and mtSSU.

Phylogenetic analysis of EF-1 α sequences clustered into ten lineages, while mtSSU sequences clustered into four lineages.

Table 1. Disease ratings produced by *F. oxysporum* isolates on 'Kabarla', taken at 8 weeks post-inoculation.

Isolate	Disease Rating (mean)	Pathogenicity rating ^y
N18421	0.6 _a	Non-pathogenic
N16004	1.4 _a	Non-pathogenic
N18419	1.4 _a	Non-pathogenic
N18582	2.0 _{ab}	Pathogenic
N15457	3.4 _b	Pathogenic
N15309	7.2 _c	Pathogenic
N18462	7.8 _{cd}	Pathogenic
N17337	9.2 _d	Pathogenic
N13581	9.6 _d	Pathogenic

Means with same subscript are not significantly different P=0.05

^y Isolates considered pathogenic if a mean disease rating >2

DISCUSSION

The pathogenicity of different isolates of *Fof* in Australia varied significantly. The level of disease varied amongst isolates, indicating heterogeneous populations of *Fof*. Two distinct VCGs were identified and corresponded to two of the ten lineages identified by partial EF-1 α . Isolates from Western Australia were distinct to those from Queensland.

ACKNOWLEDGEMENTS

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Characterisation of rice blast races in Australia

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INTRODUCTION

Rice blast caused by the fungal pathogen *Magnaporthe oryzae* is the most serious disease threat to rice production worldwide. In Australia, there have been previous records of rice blast in Northern Territory from many decades earlier and also reports of its occurrence in Queensland; the occurrence of rice blast in Western Australia was recently reported in 2011. To date rice blast remains exotic to South-eastern Australia where the majority of the Australian rice is produced, however, the previous modelling work by Lanoiselet et al. (1) highlighted the potential for rice blast to occur and threaten the rice industry in this area.

Host resistance is the most economical and environmentally friendly way to control rice blast (2, 3). However, most of the existing popular cultivars are susceptible to this disease as the resistance is rapidly overcome in the field under conditions conducive to the pathogen. This project aimed to investigate the genetic diversity and prevalence of rice blast races in Australia, and to identify rice cultivars with durable and broad spectrum resistance to rice blast under Australia conditions.

MATERIALS AND METHODS

Races of rice blast were determined using an international race differential system (3, 4) that includes eight representative cultivars (Raminad Str. 3, Zenith, NP 125, Usen, Dular, Kanto 51, Shatiao-tsao and Caloro). Ten rice blast isolates (WAC13466, BRIP15815, BRIP39772, BRIP53372, BRIP53376, BRIP59311a, BRIP58447a, BRIP15748a, BRIP25865a and BRIP53870) that were previously collected across Western Australia and Queensland were used. Eight rice cultivars (Ceysvoni, BRIRGA409, Rikuto norin 20, Shan Huang Zhan 2, Yunlu 29, NTR 426, NTR 587 and Quest) with origin from different countries were used to determine their responses to each rice blast race.

Experiments were conducted using rice seedlings at 3 to 4 leaves stage under controlled environmental conditions. There were 24 seedlings per treatment per experiment, and three repeated experiments. Disease reactions were scored at 9 days post inoculation using a 0 to 5 scale described (5) where plants with scores of 0 to 2 indicate resistant reaction and scores of 3 to 5 indicate susceptible reaction.

RESULTS

All representative cultivars showed susceptible reactions to isolates WAC13466, BRIP53372 and BRIP53870, with the disease scores greater than 3, and these three isolates belong to race IA-1. As for isolate BRIP39772, all representative cultivars showed susceptible reactions, except cv Shia-tia-tsao that was resistant to this isolate, with the disease score smaller than 2, and this isolate belongs to race IA-3. Three of the representative cultivars showed susceptible reactions to BRIP15748a, while all the other cultivars were resistant to this isolate, with the disease score smaller than 2, and this isolate belongs to race IA-63. Two of the representative cultivars showed resistant reactions to BRIP53376, while the remaining

cultivars were susceptible, and this isolate belongs to race IB-3. As for isolates BRIP15815, BRIP59311a, BRIP25865 and BRIP58447a, three of the representative cultivars showed susceptible reactions to these isolate, while the other cultivars were resistant, and these three isolates belong to race IB-59.

In relation to race IA-1, cvs Ceysvoni, BR-IRGA-HO9, Rikuto Norin 20, Yunlu 29 and Quest were susceptible, with cvs SHZ2 and NTR 426 being resistant. In terms of races IA-3, IA-63 and IB-3, all cultivars showed similar responses, with cvs Yunlu 29 and Quest being susceptible and cvs Ceysvoni, BR-IRGA-HO9, Rikuto Norin 20, SHZ2 and NTR 587 being resistant. As for race IB-59, cvs Yunlu 29 and Quest were moderately resistant while the others were resistant.

SUMMARY

This project revealed that there may be a high level of genetic diversity among rice blast isolates in Australia, with the current 10 isolates from Western Australia and Queensland belonging to five races, however, more isolates will be included to confirm the current finding.

This project also found that all cultivars showed similar responses to different rice blast isolates belonging to the same race. In general, Yunlu 29 and Quest were the most susceptible cultivars to all races while SHZ2 was the most resistant cultivar; race IA-1 was the most virulent one to all cultivars, followed by races IA-3, IA-63 and IB-3, while race IB-59 was the least virulent.

ACKNOWLEDGEMENTS

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The use of next-generation sequencing to identify sequence variation in pathogenicity-associated genes of *Fusarium oxysporum* f.sp. *cubense*

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INTRODUCTION

Fusarium oxysporum f.sp. *cubense* (*Foc*) is a serious pathogen of banana and is responsible for causing the disease, Fusarium wilt also known as Panama disease. Within *Foc*, there are four recognised physiological races, which reflect the cultivar-specific pathogenicity of an isolate (Table 1).

Several pathogenicity-associated genes have been described in other lineages of *F. oxysporum*. However, the genes regulating the host- and cultivar-specific pathogenicity of *Foc* remains largely unknown.

In this study, we sought to determine the distribution and sequence diversity of several genes previously shown to be required for pathogenicity in *F. oxysporum* in a collection of *Foc* isolates. We employed a genotype by sequence (GBS) approach to obtain low coverage whole genome sequence data for a collection of isolates representing all races of *Foc*. We hypothesised that genes involved in general pathogenicity roles would be present in all races of *Foc*. In contrast, genes involved in the host- and cultivar specific pathogenicity of *Foc* were hypothesised to have a variable distribution in the races of *Foc*.

Table 1. Race structure of *Foc*.

Gene name	Differential banana cultivars
Race 1	Lady finger
Race 2	Lady Finger, Bluggoe
Race 3	No longer valid
Subtropical race 4	Lady Finger, Bluggoe, Cavendish ¹
Tropical race 4	Lady Finger, Bluggoe, Cavendish

¹ Only able to cause disease in Cavendish when grown in subtropical climates.

MATERIALS AND METHODS

Genomic DNA was extracted from a collection of 23 *Foc* isolates representing all four races of *Foc*. Whole genome sequencing data was generated for each isolate using skim sequencing technology. A GBS analysis identified sequence polymorphisms, which were used to generate haplotypes for each candidate pathogenicity gene (Table 2).

RESULTS

All candidate pathogenicity genes were present in all races of *Foc*, with the exception of several putative effector secreted in xylem (*SIX*) genes. Of the fourteen previously described *SIX* genes (*SIX1-SIX14*), we detected nine *SIX* genes across the four races of *Foc*. Of particular interest, *SIX1* and *SIX9* were present in all races of *Foc* whilst the other seven *SIX* genes showed variable distribution across the races of *Foc*.

Table 2. Candidate pathogenicity genes investigated in the present study.

Gene name	Gene function
<i>CHS2</i>	Class II chitin synthase
<i>CHS7</i>	Chaperone-like protein
<i>FGA1</i>	G-protein α -subunit
<i>FGA2</i>	G-protein α -subunit
<i>FMK1</i>	Mitogen-activated protein kinase
<i>FOW1</i>	Mitochondrial carrier
<i>FOW2</i>	Transcription factor
<i>GAS1</i>	β -1,3-Glucanotransferase
<i>SGE1</i>	Transcription factor
<i>SIX1-SIX14</i>	Putative secreted effectors

DISCUSSION

Many of the candidate pathogenicity genes investigated in this study are known to have functions common to all *formae speciales* of *F. oxysporum* such as root adhesion and well wall degradation. As anticipated, this study has shown that all races of *Foc* carried all of these common pathogenicity genes.

In contrast, several *SIX* genes were specifically present in only some races of *Foc* but absent in other races. Previous studies have reported a similar distribution of the *SIX* genes in the races of *Foc* (1, 2). Currently, it is hypothesised that small, secreted proteins, such as the *SIX* proteins, confer the cultivar-specific pathogenicity of the races of *Foc*. As shown in this study, we expect the races of *Foc* to carry unique combinations of effector genes to facilitate the observed host- and cultivar-specific pathogenicity of *Foc* isolates.

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Unisexual mating in *Huntia moniliformis*

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INTRODUCTION

Homothallism includes a heterogeneous assemblage of mechanisms that allow sexual reproduction to occur. These have been sub-divided into discrete categories that include primary homothallism (Lin and Heitman, 2007) mating type switching and pseudohomothallism. A unique sub-class of homothallism known as unisexual reproduction has also been discovered in fungi. (Glass and Smith, 1994; Lin et al., 2005). Unisexual reproduction is also known as same-sex mating. Isolates with only a single mating type have the ability to initiate and proceed through an entire sexual cycle in culture. Preliminary characterisation of the genome of the wood colonising fungus *Huntia moniliformis* showed that it lacked the *MAT 1-1-1* gene yet this species has been characterised as homothallic. The aim of this study was to investigate the mating strategy and characterise the *MAT* locus of *H. moniliformis*.

MATERIALS AND METHODS

Cultures. Eight isolates *H. moniliformis* were used in this study.

Those isolates that produced mature ascomata were used as parental cultures from which single ascospore progeny were generated. These progeny were then visually screened for the production of ascomata and ascospore masses at the apices of ascomata, which was used as an indication of successful sexual reproduction.

***MAT* gene discovery and *MAT* locus structure.** The genes present at and associated with the *MAT* locus in *Ceratocystis fimbriata* (Wilken et al., 2014) were used in local tBLASTn searches against the draft genome assembly of *H. moniliformis* (van der Nest et al., 2014. Contigs showing hits with an E-value of 10^{-5} or less for these genes were subjected to AUGUSTUS gene prediction.

RESULTS

Cultures. Of the eight *H. moniliformis* cultures used in this study, six produced ascomata. Single ascospore progeny retained their ability to reproduce sexually in culture.

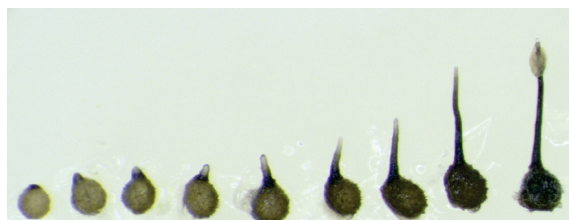


Figure 1. Microscope images of characteristic ascomata at different stages of development, from young (left) to mature (right).

***MAT* gene discovery and *MAT* locus structure.** The tBLASTn searches revealed the presence of the *MAT1-2-1*, SLA, APC and APN genes in the *H. moniliformis* genome, while neither the *MAT1-1-1* nor the *MAT1-1-2* gene was identified (Fig 1).

H. moniliformis MAT1-2 Idiomorph

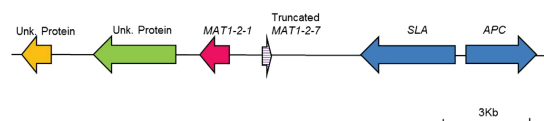


Figure 2. A schematic representation of the *H. moniliformis* *MAT* locus. Unk. Protein: Unknown protein.

DISCUSSION

The availability of sporulating cultures as well as a genome sequence for *H. moniliformis* allowed us to study the mode of sexual reproduction and to characterise the *MAT* locus in this species for the first time. *H. moniliformis*, was found to be self-fertile, with all sporulating and non-sporulating isolates possessing only the *MAT1-2-1* gene. This provides evidence for a unisexual reproductive cycle in *H. moniliformis* and is the first record of this sexual reproduction strategy in a species of the important fungal family Ceratocystidaceae. While unisexual reproduction has been previously observed in a few other ascomycetes this is the first report of the phenomenon in naturally occurring isolates where the *MAT 1-1-1* gene is absent from the *MAT* locus.

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Linking microbial ecology with plant pathology to achieve disease control and increase plant productivity

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Powerful new methodologies are available to understand the myriad of interactions of microbes with each other and with the environment in which they live. The incredible diversity of microbes that colonise plants can confer both beneficial as well as detrimental effects on plant productivity. There are now exciting opportunities to utilise the methodologies developed in the field of microbial ecology to better understand how microbial communities are assembled on plants and their effects on the plants on which they live. Investigations of the ecology of various plant pathogens such as *Xylella fastidiosa*, *Erwinia amylovora*, and *Pseudomonas syringae* have revealed novel means of disease control. Likewise, studies of the microbial taxa on healthy plants and the genes enabling their colonisation with plant responses to their presence should provide insight into how microbial communities could be established on crops to avoid disease and enable plants to approach their yield potential. While much work remains to achieve such a goal, plant pathologists will play an important role in advancing this important science.

Quantitative PCR (qPCR) and mid-infrared spectroscopic (MIR) analyses of powdery mildew on grape bunches

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INTRODUCTION

Powdery mildew (PM) (*Erysiphe necator*) is a ubiquitous disease of grapevine (*Vitis vinifera*) that can reduce yield and quality of grapes and wine. Wine made from PM-affected grapes may have elevated phenolics, flavonoids and hydroxycinnamates as well as modified sensory attributes (1).

Determining the amount of PM on grape bunches is often difficult. Visual assessment based on severity (% surface area affected on bunches) or incidence (number of affected units of total) has been used. Visual assessment of severity is subjective. Objective quantification of *E. necator* biomass was developed previously (1) but not implemented in industry.

Further developments on objective quantification of PM using qPCR and MIR spectroscopy are presented. The aims were to (a) develop a statistical model that predicts PM severity (%) based on qPCR results, and (b) test if MIR spectra provide information to allow us to identify changes attributable to *E. necator* infection.

MATERIALS AND METHODS

qPCR and MIR spectroscopy A PM-specific duplex qPCR assay using TaqMan[®] MGB-NFQ probes was designed, developed and tested using manufactured samples and naturally infected Chardonnay bunches (n=41). Homogenised healthy and fully infected berries were combined by mass (w/w) to achieve 11 manufactured PM severities. Naturally infected bunches (5.6-19 °Brix) were visually assessed using a dissecting microscope, frozen at -20°C for 8 months and then homogenised. Homogenates were used for DNA extraction, qPCR and MIR spectroscopy. Samples were scanned in the MIR range (1800-800 cm⁻¹) (Bruker Alpha). Partial least squares regression (PLS) was used for modelling and two independent models were developed using MIR data and reference data (predicted severity of PM by qPCR model and °Brix).

RESULTS AND DISCUSSION

Predicting PM severity based on qPCR results The log-ratio approach to the proportion of number of copies of amplified *E. necator* (target) and *V. vinifera* (endogenous control) fragments in manufactured samples showed that the proportion is governed by PM severity and is DNA concentration-independent. Thus, PM severity for the quantified *E. necator* biomass can be predicted based on the simplified logarithmic trend obtained for manufactured samples. Prediction takes into account the weight difference between healthy and fully infected berries, and PM severity is expressed as the ratio of fully infected to healthy berry-equivalent. By using the severity prediction model, we compared the qPCR severity predictions to the visual assessment on 41 bunches (Fig. 1). No one-to-one correspondence was present between qPCR and visual assessment since the visual assessment is surface-based while qPCR is weight-based. This lack of correspondence can be attributed to non-uniform PM density amongst berries and bias in visual surface assessment.

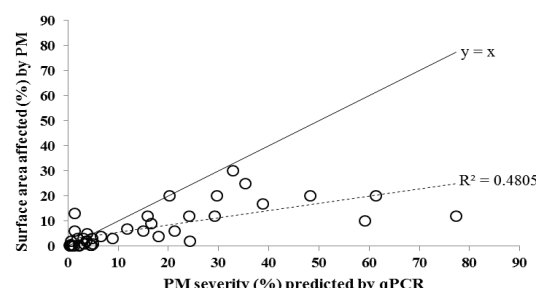


Figure 1. Correspondence between the visual assessment of the PM surface area and PM severity predicted from the qPCR analysis on 41 grape bunches.

Optimal partial least squares (PLS) loadings for the prediction of PM severity and °Brix in 16 bunches with different sugar content (14.4-17 °Brix) using MIR are given in Fig. 2. Spectral differences (indicated by arrows) were observed in the fingerprint region related to fatty acids, amides and lipids, indicating that two PLS models developed for PM severity and sugar content are independent of each other.

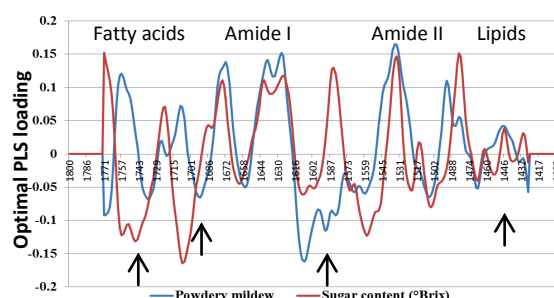


Figure 2. Spectral differences between PM and Brix

Lipids and fatty acids analyses have been undertaken to test suitability as a quantitative indicator of PM severity. The qPCR model will be validated and the usefulness of the selected biochemical markers (lipids, amides, fatty acids) utilised for PLS modelling to predict PM severity will be tested. Further work will show if MIR spectroscopy can be an objective alternative to visual assessment of PM severity.

ACKNOWLEDGEMENTS

This research was conducted with support from the Australian Grape and Wine Authority.

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Early defence responses in *Elaeis guineensis* lignin biosynthesis pathway during pathogenesis of *Ganoderma boninense*

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INTRODUCTION

Oil palm (*Elaeis guineensis*) is a valuable economic crop that is source of food security in the world. It is crucial as energy source and dietary food with beneficial nutritional values. However, oil palm has low resistance to basal stem rot (BSR) disease caused by white rot fungus, *Ganoderma boninense*. In severely affected areas in Asian continent, more than 50% of oil palms are infected with this fungus and loss in crop yield can reach up to 80% after constant planting cycles¹.

At present, there is limited understanding of oil palm defense mechanism. It is hypothesised that cell wall lignification process could serves as the first line of defense in oil palm when in contact with *G. boninense*.

Hence, this study is mainly focused on the underlying molecular and biochemical events, as well as lignin quantity and deposition patterns during pathogenesis of *G. boninense* in an *in vitro* system.

MATERIALS AND METHODS

In this study, an *in vitro* system was used to inoculate oil palm ramets (aged at one month post-rooting) with *G. boninense* (GBLS strain)². This artificial inoculation was carried out at three level of treatments (T1-absolute control, T2-wounded and T3- inoculated) and incubated under randomised complete block design for 8 days.

Then, molecular and biochemical profiles of first two targets involved in oil palm lignin biosynthesis (phenylalanine ammonia lyase (PAL) and cinnamate 4-hydroxylase (C4H)) were assessed. Genes expression levels of *EgPAL* and *EgC4H* were quantified via real-time PCR (qPCR) approach, with *EgActin* acted as the reference gene. Meanwhile, production of PAL and C4H enzymes in treated oil palm were measured spectrophotometrically through colorimetric assays.

Furthermore, lignothioglycolic acid (LTGA) assay and histochemical staining (toluidine blue-O, Maûle and phloroglucinol stains) were also carried out to evaluate the total lignin contents and deposition patterns of lignin.

RESULTS

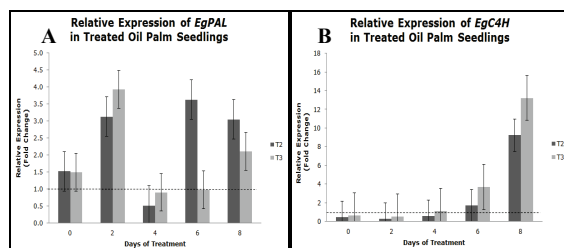


Figure 1. (A) *EgPAL* and (B) *EgC4H* expression levels of the treated oil palm within 8 days of incubation.*

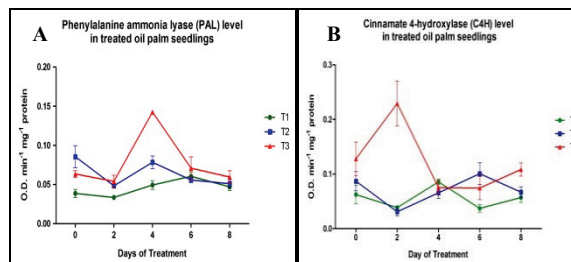


Figure 2. (A) PAL and (B) C4H enzyme levels of the treated oil palm within 8 days of incubation.*

* Standard error of mean (SEM) of triplicate readings from three rounds of experiments were represented by error bars.

For total lignin quantification, T3 samples exhibited an early decline of total lignin contents. Then, the lignin contents in T3 seedlings increased sharply and scored highest level at 93.63 TGA derivatives at 280 nm g-1 AIR fresh weights on Day 8.

Based on the histochemical staining results with three different types of dyes, it was observed that T3 samples were stained more intensive blue and brown colour by TBO and Maûle staining respectively, as compared to T1 and T2 samples.

DISCUSSION

From this study, an early induction of PAL and C4H in treated oil palm were detected in responses to wounding and infection by *G. boninense*. PAL and C4H levels were found to be highest in infected oil palm. This indicated an initial defense mechanism in oil palm was activated and resulted in the accumulation and deposition of lignin at the end of experimental period.

However, PAL and C4H levels were reduced drastically in infected samples towards later phase of this experiment, suggesting that defense responses in oil palm will eventually be suppressed by *G. boninense* through lignin modifying enzymes if prolonged infection occurs³.

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Differential accumulation of callose, arabinoxylan and cellulose in non-penetrated versus penetrated papillae on leaves of barley infected with *Blumeria graminis* f. sp. *hordei*

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INTRODUCTION

In plants, cell walls are one of the first lines of defence for protecting cells from successful invasion by fungal pathogens and are a major factor in basal host resistance. As a defence response, plants reinforce the cell wall near the site of penetration by producing a dome-shaped apposition (papilla) between the epidermal wall and the plasma membrane.

Papillae that have been effective in preventing penetration by pathogens are traditionally believed to contain callose as the main polysaccharide component. Despite previous findings, the cell wall components responsible for making papillae an effective barrier to fungal penetration are not fully understood. The overall susceptibility or resistance of a barley genotype is determined by the frequency of papillae that halt penetration effectively, compared with papillae that are ultimately penetrated by the fungus.

To improve understanding of the structural and compositional basis of papilla effectiveness, we compared the composition of papillae in susceptible cells with that of papillae in resistant cells, within the same barley line. Callose is a (1,3)- β -glucan that is easy to detect with the aniline blue fluorochrome, and we now have access to a number of polysaccharide-specific antibodies that can be used to examine papillae for other polysaccharides that have been previously overlooked.

Thus, in this study, we present a comprehensive immunohistochemical screen of the polysaccharide composition of barley papillae, using polysaccharide-specific probes (1).

RESULTS

A screen was conducted in transverse sections of barley papillae using a range of polysaccharide-specific probes, including monoclonal antibodies and carbohydrate-binding modules. The results were confirmed with two light microscopy techniques: immunofluorescence labelling allowed us to measure the fluorescence intensity in both the papilla core and the halo area, and enhanced immunogold labelling allowed us to ensure the observation of target epitopes in the papillae was not attributable to background autofluorescence associated with phenolic acid wall constituents. The data show that barley papillae induced during infection with *Bgh* contain, in addition to callose, significant concentrations of cellulose and arabinoxylan. Whole mount microscopy clearly showed that higher concentrations of callose, arabinoxylan and cellulose are found in effective papillae, compared with ineffective papillae (Figure 1). Confocal microscopy demonstrated that the papillae have a layered structure, with the inner core consisting of callose and arabinoxylan and the outer layer containing arabinoxylan and cellulose (Figure 2).

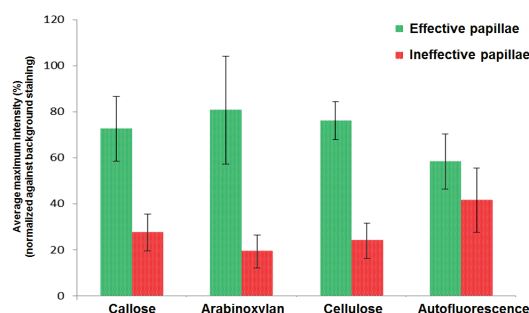


Figure 1. Relative fluorescence intensity in effective (green) and ineffective (red) barley epidermal papillae at 24 h after inoculation. Error bars, $\pm$ SD.

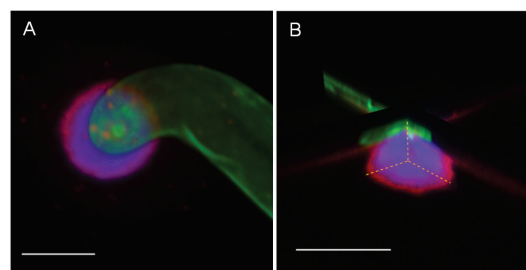


Figure 2. Confocal microscopy of polysaccharide deposition patterns in effective barley papillae formed against *Bgh* at 24 h after inoculation (hai). Labelled fungal appressoria (chitin, green) and epidermal papilla (arabinoxylan, red; callose, blue). Scale bars, 5 μ m.

DISCUSSION

In this study, we have shown that the major polysaccharides found in barley papillae are callose, arabinoxylan and cellulose. The association of arabinoxylan and cellulose with penetration resistance provides new targets for the improvement of papilla composition and for the generation of novel crop lines with greater disease resistance. The identification of genes involved in synthesising the papillae components and their role in papillae penetration resistance is currently underway.

ACKNOWLEDGEMENTS

We thank Prof. Richard Oliver for providing the *Bgh* isolate and Dr. Agatha Labrinidis from Adelaide Microscopy for her assistance. This work was supported by grants from the Australian Research Council.

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Neuronal signalling molecules as targets for green peach aphid (*Myzus persicae*) control via RNA interference

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INTRODUCTION:

Aphids are one of the most destructive agricultural pests throughout the world, including Australia. Globally, they cause significant economic losses in cultivated crops. Unlike other aphid species, green peach aphids (GPA) have a wide host range and can transmit about 110 viruses both persistently and non-persistently. Feeding is by removing host cell contents *via* a mouth stylet, and infestation has some common features with effects caused by plant parasitic nematodes. GPA populations have become resistant to many current pesticides in different regions including Australia (1). It has now become imperative to look for new and sustainable control strategies to protect crops against insect pests.

RNA interference (RNAi) is currently emerging as a stable, economical and environmentally friendly approach for insect pest control. RNAi is a sequence-specific degradation mechanism that is highly conserved among eukaryotes. In this down regulation process, double stranded RNAs (dsRNA) are cleaved by a dicer complex into small interfering RNAs (siRNAs). One strand of siRNA activates the RNA-induced silencing complex (RISC) which then degrades target mRNAs preventing production of functional proteins. Currently, RNAi has been applied successfully as a tool to study gene function and also as a control strategy for many pests where essential genes can be silenced resulting in loss of viability or lethality of target organisms. So, the efficacy of RNAi is highly dependent on selection of appropriate genes.

Although the nervous system is considered an effective target for chemical control, little attention has been paid to this system as a possible target for biological control (2). Genes regulate the nervous system via production of neurosignalling molecules (NSMs) which include neuropeptides, their receptors and biogenic amine receptors. The current study was undertaken to evaluate the effect of silencing any of these genes on the reproduction and mortality of GPA and to provide proof-of-concept of RNAi as a control strategy for GPA.

MATERIALS AND METHODS

Identification of target NSM genes for RNAi in GPA: Target genes were identified from a transcriptome sequenced from mixed stages of GPA. Comparative genomics tools were used to identify transcripts encoding NSMs using genes from databases such as NCBI, Flybase, Aphidbase and Wormbase.

Artificial feeding bioassay or *in vitro* RNAi: DsRNA (2µg/µL) for six genes with controls (dsRNA of GFP and no dsRNA) were each mixed with 30% sucrose with 0.02% Neutral Red and separately fed to five 3rd instars of GPA per treatment. After 24 hours, fed aphids were observed for changes in behaviour and later transferred to canola plants to assess effects of silencing the genes on longevity and reproduction.

RESULTS AND DISCUSSION:

Changes in aphid behaviour after dsRNA feeding: The presence of neutral red in the stylet and gut indicated all treated aphids fed on the artificial diet. DsRNA feeding of five of the genes appeared to have affected the behaviour of aphids (Table 1).

Table 1: RNAi phenotypes of six GPA NSM genes.

Gene	RNAi phenotype after 24 h	
1	100% extremely paralysed	
2	No mortality observed but all stressed	
3	60% inactive with less movement	
4	No obvious phenotype	
5	20% mortality; could not moult	
6	20% mortality; could not moult	
GFP	100% alive and active	
No dsRNA	100% alive and active	

RNAi effect of NSM genes on GPA longevity and reproduction on canola plants: Aphids fed dsRNA of Genes 4 and 5 had reduced rate of reproduction ($p < 0.05$, Fig.1A). RNAi of Gene 1 had 100% lethal effect and feeding on dsRNA of Genes 3, 4, 5 and 6 resulted in significant reduction in longevity ($p < 0.05$, Fig. 1B).

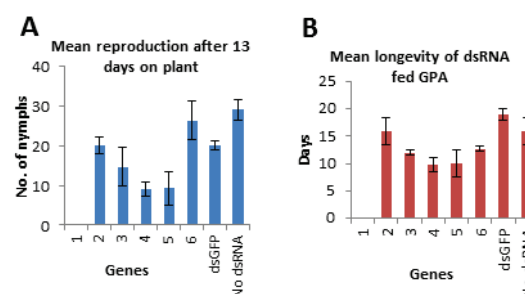


Fig 1: Effect of dsRNA feeding on GPA survival and reproduction.

Feeding GPA nymphs with dsRNA resulted in silencing of the genes as evident from the phenotypic abnormalities observed indicating the genes are vital for development. For three genes, the longer term effect of silencing manifested in reduced reproduction and longevity on canola plants. The results indicate that *in vitro* RNAi is a good functional analysis tool to identify target genes (eg. NSMs) for pest control. These genes can then be used to engineer host plants of economic importance to deliver silencing triggers targeting essential genes to control their populations and to reduce damage caused by GPA and in general insect pests *via* the RNAi approach.

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Endophytic *Trichoderma* in the disease management of *Ganoderma* disease of oil palm—deciphering the mechanisms

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INTRODUCTION

For more than half a century, severe economic losses continued to be experienced by the South East Asia oil palm industry due to *Ganoderma* basal stem rot. A number of approaches have been employed in managing the disease which includes cultural practices, chemical and biological control. This paper attempts to decipher the potentials of a well-known antagonist, *Trichoderma* in controlling this disease. We were the first to report the isolation of this genus as an endophyte from the roots of oil palm. The initial screening of these potential endophytic *Trichoderma* was based on *in vitro* and subsequently tested at nursery. Two *T. virens* isolates; 7b and 159c were selected and subjected to the profiling of antifungal compounds released and physical mechanisms responsible in the inhibition against *Ganoderma*. The physical attributes of the isolates were assessed via scanning electron microscope (SEM) and siderophore assay and the chemical mechanism was assessed through thin layer chromatography (TLC) followed by gas chromatography mass spectrophotometry (GCMS) which led to the identification of antifungal compounds.

MATERIALS AND METHODS

Fungal source *Ganoderma* was obtained from GanoDROP lab from Malaysian Palm Oil Board. Endophytic *Trichoderma* were isolated based on Evans (1). Cultures were maintained in potato dextrose agar (PDA).

Screening Isolated *Trichoderma* isolates were tested against *Ganoderma* via dual culture and poison agar assays. Verification at nursery was also carried based on Sundram *et al.*, (2).

Physical and chemical properties Mycelia of *Ganoderma* at the inhibition zone was excised and subjected to scanning electron microscope (SEM). *Trichoderma* cultures were grown in liquid cultures before subjected to separation using three different solvents (hexane, ethyl acetate and butanol). Extracts were assessed using thin layer chromatography (TLC) and gas chromatography mass spectrophotometry (GCMS). The extracts were also assessed using well diffusion for inhibition verification. Siderophore detection was also carried out.

RESULTS

Screening Selected *Trichoderma* isolates were tested at nursery. Isolates 7b and 159c gave significant disease control. Both isolates were identified as *T. virens* using ITS primers.

Physical and chemical properties Dual culture results as showed in Table 1 with significant inhibition by 7b and 159c. The antifungal activity of hexane extract was comparable with the fungicide through disc diffusion assay. SEM images as seen in Figure 1 shows the morphological defects by the extracts of *T. virens* 7b. Siderophore assay found 7b aggressively chelating iron.

Table 1. Inhibition of *Ganoderma boninense* by extracts of culture filtrates of *Trichoderma virens* 7b

Extract	Percentage of inhibition growth (PIRG), %
Crude extract	41.46 ± 4.119 ^a
n-hexane extract	62.60 ± 6.414 ^b
Benlate	75.55 ± 2.569 ^c

^a Percentage of inhibition growth was calculated using blank DMSO as a negative control. Each value is the mean percentage of three experiments ± standard deviation. Means within the same column followed by the superscript of same letters are not significantly different at P<0.05 with LSD test.

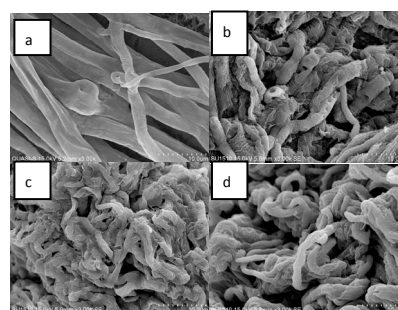


Figure 1. Scanning electron micrographs (10.0 µm) showing the region of the inhibition zone where the growth abnormalities of *G. boninense* were observed. The mode of inhibition against *G. boninense* was observed by treatment of different extracts. (a) Control hyphae, (b) Crude extract, (c) Hexane extract, (d) Benlate.

DISCUSSION AND CONCLUSION

Endophytic *Trichoderma* was found to be effective against *G. boninense* through various mechanisms. The current work provides understanding of the non-polar secondary metabolites released by endophytic *Trichoderma*. The results suggested that non-polar compounds (Phenylethyl alcohol, PEA) were responsible in the suppression of *G. boninense* as crude extract (50 mg/ml) with higher antifungal activity when compared to polar extract. The highly deformed hyphae resulted from the study verifies the presence of antifungal agents in hexane extract that was responsible for the suppression of *G. boninense* growth. Work is currently on-going to commercially produce a product containing *T. virens* as the active ingredient.

ACKNOWLEDGEMENTS

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Histopathology of *Stagonosporopsis tanacetii* infected pyrethrum seed

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INTRODUCTION

Pyrethrum (*Tanacetum cinerariifolium*) belongs to the family Asteraceae, and is grown commercially in Australia for the production of natural insecticidal pyrethrins. The main source of the pyrethrins is the achenes and flowers (Pethybridge et al., 2008). Ray blight caused by *Stagonosporopsis tanacetii* infects flower stems and flowers and is considered as one of the main limiting factors of pyrethrum production (Vaghefi et al., 2012). Infected pyrethrum seed has been considered as the primary source of inoculum of *S. tanacetii* and as such epidemics of ray blight disease have been linked to seed infection (Pethybridge et al., 2008). In the previous study, although it was assumed that infected seedlings developed from the infected seed, there was no research into the colonisation of seed tissue within the seed. Hence, a study was undertaken to determine the extent of seed colonisation by *S. tanacetii* and the effect this had on germination.

MATERIALS AND METHODS

Germination of seed. Two seed groups, 10 naturally infected pyrethrum seeds (var. BR1) and 10 steam sterilised seeds (var. BR1) were placed on petri dishes with sterile moistened filter paper and incubated at 25 °C. Seven replications were maintained for each seed group. After 2 weeks the percentage germination was recorded.

Dissection of seed and mycoflora of seed parts. Seeds (40) from the naturally infected seed lot were dissected to separate outer and inner seed coat, and the embryo using a sterile needle and tweezers under a dissecting microscope. Seed parts were then sterilised with 1% sodium hypochlorite for 2 min, placed onto 1% water agar and incubated in the dark at 20 °C for 2 weeks. Mycelia from the seed parts were cultured on V8 medium to confirm the presence of *S. tanacetii*.

Histopathology of seed. Five infected and 5 healthy seeds were fixed with FAA, dehydrated with 5 increasing concentrations of ethanol, embedded in paraffin, microtome sectioned (5-7 µm) with a rotary microtome then stained with Johansen modified quadruple stain. Sections were observed under a light microscope (Leica DM 6000 B) and the images were taken using a Leica DFC 450 C camera.

RESULTS AND DISCUSSION

Germination rate of steam sterilised seed (100%) was significantly higher than the naturally infected seed (70%). Seeds that did not germinate were infected by *S. tanacetii* thus proving that *S. tanacetii* could be an important pre-emergent pathogen. Previous research had also shown that the incidence of *S. tanacetii* in commercial seed lot was about 28% (Pethybridge et al., 2006). Both the outer and inner seed coat were infected with *S. tanacetii*; however no infectious hyphae were detected in the embryo. Similarly, using microtome thin sections (5-7 µm) infection hyphae were seen in outer seed coat (Fig 1 a), 1st inner sclerenchymatous seed coat and in between the outer and inner seed coat but not in the embryo of infected seed. Pyrethrum seed was composed of an outer coat with 2-3 layered thick sclerenchyma cells (35-65 µm), 1st inner seed

coat with single layered sclerenchyma cells, 2nd inner seed coat with single layer parenchyma cells (combined inner coat 8.5-25 µm) and embryo with homogenous parenchyma cells (350-675 µm). There was a gap (1.5-27 µm) found between seed coat and embryo which may have acted as a physical barrier to protect the embryo from *S. tanacetii* infection in the inner seed coat (Fig 1 b). Similarly, Lee et al. (1984) showed that the fungus *Didymella bryoniae* was located on and in the seed coat of cucumber seed (*Cucumis sativus*) but not in the embryo.

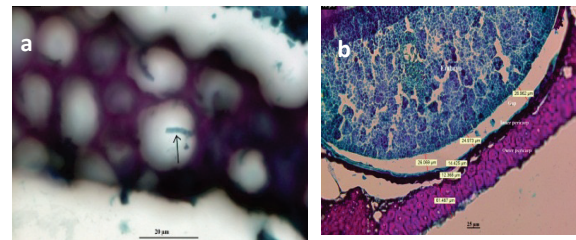


Fig 1. Histopathology of infected pyrethrum seed (a) mycelia in the outer sclerenchymatous seed coat (b) the structural composition of seed with gap between pericarp and embryo. Arrow indicates infection hyphae.

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Seed used in this research was supplied by Botanical Resources Australia Pty Ltd.

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Efficacy of conventional and biological fungicides for managing powdery mildew on two muskmelon varieties with different genetic susceptibility to the disease

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INTRODUCTION

Powdery mildew (PM) of cucurbits, caused by *Podosphaera xanthii*, is a common and often serious disease in many regions of the world. PM occurs annually in Arizona muskmelon, watermelon, and mixed melon plantings, and many planted varieties are susceptible to the disease. Several conventional fungicides and biofungicides are registered for use in the United States for managing PM on cucurbits. Efficacy of biofungicides is generally lower than that of conventional fungicides (1), especially in Arizona where disease pressure on muskmelon plantings is often severe (2). Effective management of PM on a susceptible muskmelon variety when disease severity is high cannot be achieved with biofungicides alone (2). The purpose of the following study was to compare the efficacy of some conventional fungicides and biofungicides for managing PM on a susceptible muskmelon variety compared to one with some genetic resistance to disease.

MATERIALS AND METHODS

Field trials were conducted in 2013 and 2014 at The University of Arizona Yuma Agricultural Center. Two muskmelon varieties, Olympic Gold (susceptible to PM) and Navigator (with some genetic resistance to PM), were seeded in mid-March on raised beds with 204 cm between bed centers. Each tested fungicide was applied alone to five replicate plots, each consisting of a 7.6 m length of bed, arranged within the trial in a randomised complete block design. Each fungicide was applied at the top labelled or proposed labelled rate, initially in mid-May and continuing at 7 to 10 day intervals to crop maturity in mid-June. Four and three applications of each fungicide were made in the 2013 and 2014 trials, respectively. PM was first observed in each trial at the end of May. At crop maturity, disease severity was assessed by collecting 10 leaves from each replicate plot near the base of plants at a distance interval of approximately 0.5 m and estimating the proportion of the upper and lower leaf surface area covered with PM. The following disease severity rating system was employed, with a disease rating of 0, 1, 2, 3, 4, or 5 corresponding to respective leaf surface areas covered with PM of 0, 5 to 10, 15 to 25, 30 to 50, or 55 to 100%.

RESULTS AND DISCUSSION

Table 1 shows the efficacy of some tested conventional fungicides and biofungicides against PM on muskmelons. Disease severity in the 2013 trial was low, as shown by the low level of PM on the nontreated susceptible variety Olympic Gold. For plants treated with the biofungicides *Bacillus pumilus* and *Streptomyces lydicus* as well as the conventional fungicide trifloxystrobin, the severity of PM on resistant muskmelon Navigator was significantly lower compared to disease severity on the susceptible variety Olympic Gold. There were no statistical differences in PM severity among the two muskmelon varieties treated with other fungicides. Disease severity in the 2014 trial was high, with significantly higher levels of PM on nontreated Olympic Gold compared to resistant Navigator. The severity of PM on Navigator plants was significantly lower compared to disease severity on the susceptible variety Olympic Gold

when treated with the biologically derived Tea Tree Extract and *Bacillus amyloliquifaciens* as well as the conventional fungicide boscalid. No significant differences in PM severity among the two muskmelon varieties treated with other fungicides were observed.

Data from these studies suggest that the tested biologically based fungicides can be effective tools for management of PM on muskmelon varieties possessing some genetic resistance to the disease, thus making available to growers the advantages of these types of disease control products.

Table 1. Effect of fungicides against powdery mildew on susceptible (Olympic Gold) and resistant (Navigator) muskmelons.

Fungicide	Powdery mildew rating	
	Olympic Gold	Navigator
<u>2013 field trial</u>		
Nontreated	1.6 a	1.5 a
<i>B. pumilus</i>	1.6 a	0.8 bc
<i>S. lydicus</i>	1.6 a	0.7 cd
Trifloxystrobin	0.9 b	0.4 e
Pyraclostrobin	0.6 d	0.8 b-d
Quinoxifen	0.1 fg	0 g
Triflumizole	0.1 g	0.1 g
Myclobutanil	0.1 fg	0.3 ef
Wettable sulfur	0 g	0 g
<u>2014 field trial</u>		
Nontreated	3.9 a	1.3 b
Tea tree extract	0.9 c	0.2 ef
<i>Bacillus amyloliquifaciens</i>	0.8 c	0.1 f-h
Boscalid	0.8 c	0.3 de
Myclobutanil	0.4 d	0.3 de
Tetraconazole	0.3 de	0.2 de
Triflumizole	0.3 de	0.2 de
Quinoxifen	0 h	0 h

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The effects of a novel Chrysovirus on asexual and sexual spore production in *Aspergillus* species

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INTRODUCTION

Over the last couple of decades interest in mycoviruses has been mostly driven by interest in their potential as biological control agents of plant pathogens. Studies have largely focussed the effects of viruses on the virulence of fungal pathogens (1) with much less attention being given to the effects of viruses on fungal life cycles, such as changes in asexual and sexual spore production. A novel chrysovirus discovered in *Aspergillus thermomutatus* illustrates the effects viruses can have on sporulation with potentially significant effects on the fungal life cycle.

MATERIALS AND METHODS

Virus sequencing and phylogenetic analysis. Viruses were detected by dsRNA purification and sequence obtained by random primed RT-PCR, cloning and Sanger sequencing. Nucleotide sequences were assembled using Geneious (2) and the most similar sequences determined by BLAST. Nucleotide and amino acid sequences were aligned using Geneious and phylogenetic trees were constructed using MEGA 6 (3).

Sporulation rate comparison. Virus free and virus infected, genetically identical, lines of *Aspergillus* isolates were obtained by cycloheximide treatment to remove dsRNAs in *A. thermomutatus* and by protoplast transfection to generate virus infected lines of *A. fumigatus*, *A. niger* and *A. nidulans*. Cultures were grown at 20°C and 37°C on PDA plates.

Transcriptome analysis. Total RNAs were extracted using a Spectrum™ plant total RNA kit (Sigma-Aldrich) and sequenced using the Illumina HiSeq platform. Sequence data analysis was performed using the Galaxy web-based platform (4).

RESULTS

Virus sequence analysis. A virus associated with poor asexual sporulation in *Aspergillus thermomutatus* (AthCV1) consisted of four dsRNA segments, each containing a single ORF (Fig. 1). The longest segment (3589 nt) encoded a putative RNA-dependent RNA polymerase (RdRp), and the second longest (2773 nt) encoded putative coat protein (CP), whereas each of the two other segments (2676 nt and 2514 nt) encoded for hypothetical proteins. BLAST and phylogenetic analysis showed that *Botryosphaeria dothidea chrysovirus 1* was the closest virus (57% aa similarity). Phylogenetic analysis placed the two viruses in the same clade within the genus *Chrysovirus*.

Sporulation rate comparison. Virus free *A. thermomutatus* produced large numbers of conidia but no ascospores at both 20°C and 37°C, whereas the virus infected line also produced large numbers of ascospores at both temperatures and ten-fold fewer conidia at 20°C. Both virus free and virus infected *A. fumigatus* produced only conidia at both temperatures, with a ten-fold reduction in sporulation in the virus infected line. Conidial production was significantly reduced in the infected lines of *A. nidulans* and *A. niger* at 37°C with a two-fold increase in ascospores in *A. nidulans* infected line at both temperatures. *A. niger* also produced significant numbers of sclerotia at 37°C.

Transcriptome analysis. Virus infection had an effect on gene expression in all four *Aspergillus* species, including the expression of genes known to be involved in sporulation.

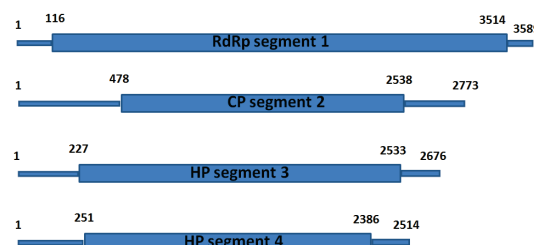


Figure 1. Schematic representation of the four AthCV1 genome segments: RdRp (1), CP (2), and two hypothetical proteins of unknown function (3 & 4).

DISCUSSION

A new virus species in the genus Chrysovirus was detected in *A. thermomutatus* and was shown to replicate in transfected *A. fumigatus*, *A. niger* and *A. nidulans*. The virus was provisionally named *A. thermomutatus chrysovirus 1* (AthCV1).

AthCV1 had significant effects on both sexual and asexual reproduction of the four *Aspergillus* species, demonstrating that mycoviruses can have significant effects on the life cycles of their host fungi in addition to effects on pathogen virulence.

ACKNOWLEDGEMENTS

We acknowledge the Faculty of Science, The University of Auckland for funding this research and Dr David Holland for providing *Aspergillus* cultures.

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Improving soil organic carbon levels enhances suppression of root lesion nematode in sugarcane soils

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INTRODUCTION

Lesion nematode (*Pratylenchus zae*) is one of several important soilborne pests of sugarcane. Recent work has shown that soil just under the trash blanket is much more suppressive to the nematode than soil from deeper in the profile (1). Since suppressiveness is driven by carbon inputs from crop residues and roots (2), this study aimed to determine the soil carbon levels required to achieve useful levels of suppression.

MATERIALS AND METHODS

Samples were collected at three depths in the soil profile [surface (0-1 cm); upper (1-2.5 cm); and lower (5-10 cm)] from sugarcane fields with sandy loam soils (34 samples) and clay loam soils (15 samples).

Nematodes were extracted from each sample using standard methods and plant-parasitic and free-living nematodes were counted.

Sugarcane was planted in 1L pots filled with each soil and pots were inoculated with 500 *P. zae*. Plants were grown in a greenhouse for 8 weeks and then roots were carefully removed and placed on a nematode extraction tray. After 8 days, nematodes were counted and roots were dried and weighed.

A number of chemical and biological parameters were assessed in rhizosphere soil. Nematodes were extracted and counted; microbial activity was estimated by measuring the rate of hydrolysis of fluorescein diacetate (FDA); CO₂ respiration was measured using the commercial Solvita® CO₂-burst protocol; and total carbon and labile carbon were measured using standard techniques. Relationships between these parameters were assessed using regression analysis.

RESULTS

In both groups of soils, the nematode community and the soil carbon status varied markedly with depth (Table 1). Soil C levels and populations of free-living nematodes were consistently high in surface soils and declined with depth, whereas the reverse was true for *P. zae*.

Table 1. Total organic carbon (TOC) and numbers of free-living nematodes and *P. zae* (mean ± SE) in N samples collected at different depths in the soil profile from fields with sandy loam and clay loam soils (top and bottom rows, respectively).

Depth	N	FLN	Lesion	TOC
Surface	14	14723±1694	2±1	1.42±0.04
Upper	9	3580±603	68±13	1.08±0.04
Lower	11	1723±138	161±31	1.05±0.05
Surface	8	8955±1431	0±0	4.09±0.54
Upper	3	3953±557	97±35	2.47±0.10
Lower	4	3095±437	100±29	2.01±0.03

Parameters that provide a broad indication of a soil's biological status (rate of hydrolysis of FDA, CO₂ respiration

and total free-living nematodes) were significantly and positively related to each other in both soil types. All three parameters were also significantly and positively related to total C and labile C.

Total C and labile C were strongly related to each other. Lesion nematode populations were negatively related to total C and labile C (whether measured as nematodes/pot or nematodes/g root). In the sandy loam soil, the regression equation indicated that an increase in soil C from 1 to 1.5% reduced populations of *P. zae* from 21,320 to 4,095 nematodes/g dry weight of root (Fig. 1A). Soil C levels were higher in the clay loam soils, but a 0.5% increase in soil C levels (from 2 to 2.5%) decreased populations of *P. zae* from 11,500 to 7,380 nematodes/g dry weight of roots (Fig. 1B).

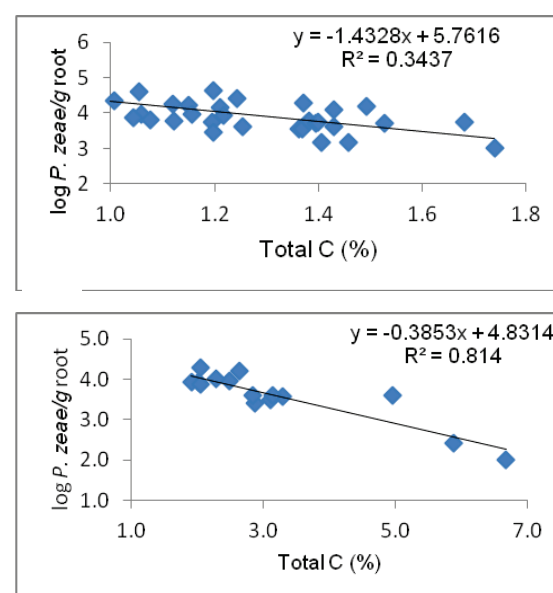


Figure 1. Relationship between soil C levels and numbers of *Pratylenchus zae* in sugarcane roots grown in sandy loam soils (A) and clay loam soils (B).

DISCUSSION

Soil carbon plays a central role in influencing the physical, chemical and biological health of soil. This study shows that one of the biological benefits that is obtained from improving soil carbon levels is enhanced suppression of root lesion nematode.

ACKNOWLEDGEMENTS

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Integrated treatment of citrus fruit for the control of *Penicillium digitatum*, causal agent of citrus green mould: a revision of common praxis

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INTRODUCTION

Globally, loss of citrus fruit as a result of decay caused by the postharvest pathogens, *Penicillium digitatum* (green mould), *P. italicum* (blue mould) and *Geotrichum citri-aurantii* (sour rot) has been shown to exceed 40% in export crops (1). Postharvest damage as a result of infection by these three pathogens is usually controlled using fungicides such as imazalil or guazatine (2). However, difficulties with accurate application of the fungicides under ideal conditions, resistance problems, cost, and objections to fungicide residues by international markets, have all combined to limit the use of postharvest fungicides. The need for environmentally and consumer-friendly alternative control measures, which are effective and affordable, has therefore assumed priority. Prior research suggests that the application of a potassium silicate fertiliser in the orchard prior to harvest, combined with novel treatments in the packhouse, may provide a competitive alternative to fungicides (3). Soluble silicon fertiliser applications pre-harvest has also been shown to improve the ability of citrus fruit to withstand chilling injury (pers. Comm.). This is of particular importance where fruit is exported to markets where cold sterilisation is a requirement (21 days at -0.5°C), to kill fruit fly larvae (4). The primary objectives for this study were to evaluate the combined effects of hot water plus a biological control agent against green mould of citrus, and to test the effects of preharvest silicon treatments on chilling injury prevention in lemons.

MATERIALS AND METHODS

Preharvest: Fruit trees (lemon, Navel, Valencia) were treated with potassium silicate (liquid form and granular form) plus controls, once monthly for a period of 18 months. Silicon uptake in fruit flavedo, rind and leaves was evaluated. Chilling injury tolerance in fruit and resistance to disease in fruit was evaluated.

Postharvest: Fruit picked from preharvest treatments were subjected to hot water treatments (58-68°C for 5, 10, 15 and 20s), then either treated with a yeast biocontrol agent (B13) or not treated, then inoculated with spores of *P. digitatum* (1x10⁶ spores/ml). Disease development was evaluated.

RESULTS AND DISCUSSION

Hot water treatment: Hot water treatment of fruit at 60°C x 20s, 64°C x 5, 10, 15 and 20s, followed by treatment with B13, resulted in the lowest number of diseased fruit, 2 weeks post-treatment. Fruit showed reduction in disease development ranging from 95-98%. This was consistent for both Navel (Figure 1) and Valencia (Figure 2). Furthermore, there was little variation as a result of the treatment times, i.e., exposure of fruit for 10, 15 or 20 seconds did not differ significantly between treatments (confirmed using Duncan's Multiple Comparison analysis). This could imply that temperature is the principal determining factor of the treatments, and that the heat shock event takes place very rapidly, at the correct temperature. This heat shock reaction could then eliminate any infection that may be setting in, and the B13 treatment which follows serves as a

preventative measure against infection during postharvest processing outside of the packhouse. The use of B13 + hot water treatments can rival treatment of fruit with Imazalil alone. Results were not statistically different between combined treatments and the use of Imazalil alone.

Chilling injury: Fruit were rated for injury according to a rating scale derived from Obenland et al. (5). Fruit picked from trees that received preharvest silicon treatments, either in liquid form or granular form showed superior tolerance to chilling injury (Figure 3). Fruit picked from trees receiving either potassium sulphate or plain water succumbed to chilling injury far quicker and far more severely, rendering them un-marketable in a real-time export situation. Furthermore, silicon uptake was assessed in the flavedo of the fruit using electron scanning microscopy (Table 1). Results showed that silicon is indeed taken up by the plant and deposited to fruit, and it is postulated that this increased level in silicon in the flavedo is able to confer chilling injury protection. Preharvest silicon treatments also appeared to predispose the fruit to dehydration tolerance.

Table 1. Elemental composition of lemon fruit flavedo, with emphasis on silicon.

Treatment	Major elemental composition of fruit flavedo (%)					
	Si	C	O	P	K	Ca
KSil-Liq	1.30	49.89	61.22	0.06	7.11	0.25
KSil-Gran	0.76	48.72	62.91	0.02	6.43	0.41
PotS	0.15	50.64	48.01	0.06	0.72	7.00
Control	0.07	49.89	48.92	0.06	0.62	3.85

ACKNOWLEDGEMENTS

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Suppression of *Fusarium* crown rot in wheat by endophytic actinobacteria

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INTRODUCTION

Fusarium Crown Rot in cereals, caused by the fungus *Fusarium pseudograminearum*, reduces both the yield and quality of cereals, costing growers an estimated \$80M for wheat and \$18M for barley annually in Australia alone [1]. Management strategies to control Crown Rot have been largely unsuccessful to date. Fungicides used as seed dressings provide little protection. Plant breeding has produced more tolerant varieties but none of these show effective disease resistance. Furthermore, after maturity *Fusarium* grows and survives in the stubble regardless of the level of resistance/tolerance of the wheat variety in the living phase of the crop increasing the risk of infection in the following crop. This project is exploring the development of biological control agents for Crown Rot.

MATERIALS AND METHODS

A collection of endophytic, plant growth promoting actinobacteria were isolated from the roots of wheat plants that were selected by asking growers to indicate areas of their farms that consistently out-performed other areas in terms of plant health and yield. The antifungal activity of the isolates was tested in *in-vitro* competition tests against *F. pseudograminarium* on agar plates. The ability of the antifungal isolates to suppress disease *in-planta* was then tested in a soil free, *in-vitro* test by germinating isolate coated seeds on paper towel rolls, infecting them with *F. pseudograminarium* at the two leaf stage and assessing the difference in disease score (% stem browning) relative to untreated seeds after a further two weeks of growth. A subset of the isolates were identified by extracting and sequencing the 16s ribosomal RNA genes and comparing them to the Genbank database.

RESULTS AND DISCUSSION

Many of the isolates showed varying degrees of pathogen suppression on plates, with several completely inhibiting the growth of the fungus (Figure 1 and Table 1). The isolates with the strongest antifungal activity from the plate tests also showed significant disease suppression in soil-free small plant assays (Figure 2).

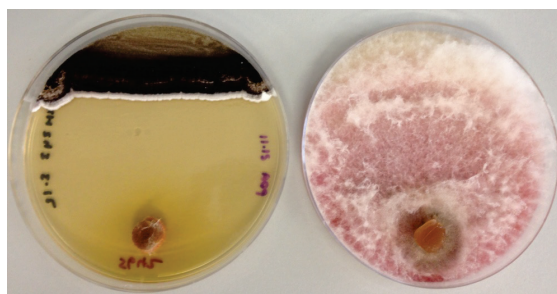


Figure 1. *In vitro* suppression of *F. pseudograminearum*. On left, *Streptomyces* sp. (MH243—top of plate) inhibited growth of *F. pseudograminearum* (bottom of plate) compared with control plate (on right) with *F. pseudograminearum* alone.

Sequencing of the 16s rRNA gene revealed that the two isolates with the strongest antifungal activity were *Streptomyces* species. Other isolates with lesser antifungal

activity belong to the *Rhodococcus*, *Micromonospora* and *Microbispora* genera.

Table 1: *In vitro* antifungal activity of actinobacteria against *F. pseudograminearum* strains CS5642, CS5834 & CS3427

Actinobacteria Isolate	Suppression zone (mm)			Genus
	CS5642	CS5834	CS3427	
MH243	32	44	18	<i>Streptomyces</i>
MH71	25	27	35	<i>Streptomyces</i>
MH191	12	4	15	<i>Streptomyces</i>
MH192	11	18	18	<i>Streptomyces</i>
MH60	9	3	4	<i>Streptomyces</i>
8a	7	2	4	<i>Streptomyces</i>
MH99	0	0	0	<i>Micromonospora</i>
MH184	0	-	-	<i>Microbispora</i>
66b	0	25	0	<i>Rhodococcus</i>

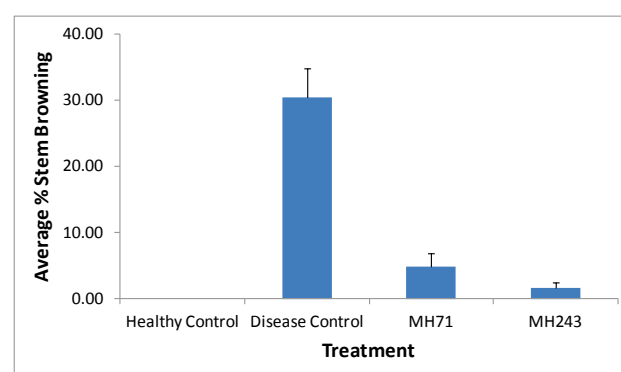


Figure 2. Average percentage of stem browning observed for plants (wheat cv. Tamaroi) inoculated with actinobacteria compared with healthy (negative) and disease controls plants infected with *F. pseudograminearum* CS5642.

The antifungal isolates have several qualities that make them promising as biocontrol agents. They are easy to culture in the lab and to introduce to plants as a seed coat, they were shown to readily recolonise the intercellular spaces of wheat roots from a seed coating and they are also spore formers giving them an additional mechanism to survive adverse environmental conditions.

ACKNOWLEDGEMENTS

The authors thank Dr Louise Thatcher for advice on DNA sequencing and bioinformatic analysis.

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Biological control of Monilinia and Botrytis in Tasmanian cherries

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INTRODUCTION

Cherry growers in Tasmania widely consider that crops suffer from brown rot (*Monilinia* spp) but that grey mould (*Botrytis cinerea*) is not a problem. Diagnostic surveys by the author have found that crops are badly infected in cool wet seasons by both *Botrytis cinerea* (grey mould) and brown rot (*Monilinia laxa*) in approximately equal proportions. *Monilinia fructicola* (also known as brown rot) is also recorded in Tasmanian cherries, but *M. laxa* appears to be more common. Both diseases infect the flowers to create latent infections which cause decays as the fruit mature, either in the final weeks of ripening or in post harvest storage. Both diseases produce masses of conidia and can spread very quickly among ripening fruit.

Chemical fungicides are used to manage the disease and a typical program may include 16 sprays per season. Residues limit use for late season control and an environmentally friendly disease control measure is urgently needed for management, sustainability and consumer expectations.

Three *Trichoderma* races all collected from southern Tasmanian vineyards and identified for ability to suppress *B. cinerea* in grapes (Metcalf & Scherer, 2005), and as antagonists of *M. laxa* in culture experiments, were screened for ability to suppress *B. cinerea* and *M. laxa* in cherries. The initial target was reduction of latent infection of flowers as reduction in latent infection would make a substantial contribution to disease management.

METHODS

The trial was conducted in 2011/12 season at Plenty, Tasmania (cv: Sweatheart), and employed a randomised complete block design with ten replicates of each treatment, each replicate being a single adult tree bearing around 500 fruit. The trial site had been subject to the growers conventional chemical program prior to trial establishment at flowering.

T. koningii Td67 (Colonizer™) (Metcalf & Scherer, 2005), *T. harzianum* Td81b (Antagonizer™) or *Trichoderma* spp. Td84 (Solstice™) spore powders (Metcalf Bio Control) containing 5×10^{10} conidia applied at 300L/ha by knapsack sprayer targeting only the flower zone. The sprays were applied twice during flowering, a further application was made three days before commercial harvest allowing little/no time for effect. The trial included comparison to the growers commercial fungicide program which included a dozen sprays variously of copper oxychloride, captan, procymidone and iprodione.

The trial was assessed by counting the total number of fruit in each replicate and the number infected immediately prior to harvest.

RESULTS

The disease incidence at harvest (Figure 1) shows that both *M. laxa* and *B. cinerea* were present in the crop. The incidence of *M. laxa* was 3.49% (Figure 1) in the untreated control, the best reduction in infection was provided by *T. harzianum* Td81b which reduced infection to 0.76% (sig LSD0.05=0.98) and comparable to the levels of control to the chemical program. The incidence of *B. cinerea* in the

untreated control was 0.73% (Figure 1) and the incidence of infection in *T. harzianum* Td81b treated fruit was 0.13% (non sig P=0.071).

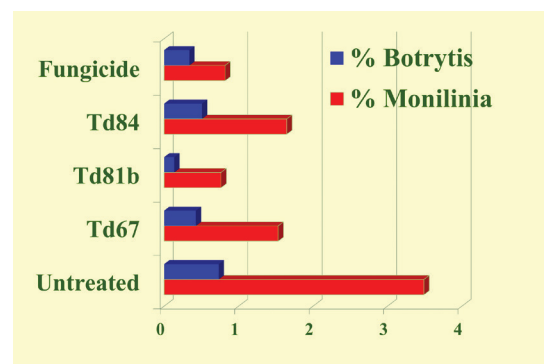


Figure 1: Incidence of *B. cinerea* and *M. laxa* infection among fruit treated at flowering with *Trichoderma* spp or a chemical program (lsd0.05 Bot ns, Mon 0.97).

DISCUSSION

The reductions in infection are encouraging and offer significant potential to suppress grey mould and brown rot which have proven very challenging diseases to manage. In this time after trial initiation the grower applied 11 chemical fungicide sprays to the surrounding crop in comparison to the three applied to the trial (the last of which was too late to derive benefit), suggesting that there is significant potential to save costs.

The presentation will detail the commercialisation pathway that followed this experiment.

ACKNOWLEDGEMENTS

Thanks to Nick Owens and Tim Reid of Reid Fruits for supporting this trial.

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Novel green manure: control of specific replant disease on the rosaceous ornamental tree *Sorbus aucuparia*

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INTRODUCTION

Specific Replant Disease (SRD) is a chronic global issue that is reported to affect broadleaf species within the Rosaceae by reducing their growth, vigour, and may result in death. The loss of rosaceous trees in the UK alone is estimated to be £1 million per year. Subsequent to 1993, broad spectrum fumigant, methyl bromide was successfully used as a control for this disease (1). This chemical was revoked as it was declared a stratospheric pollutant. The use of an Integrated Pest Management approach is becoming increasingly important, with an emphasis in the reduction of chemicals used. This research looks into the potential of a Novel Green Manure (NGM) known for having antimicrobial properties. Previous *in vitro* experiments by the author demonstrated the fungistatic properties of this material ($p < 0.001$) against *Rhizoctonia solani* and *Pythium ultimum* ($p < 0.05$).

MATERIALS AND METHODS

Four hundred *Sorbus aucuparia* (Rowan) trees were amended with a Novel Green Manure (NGM) and the ADAS bioassay used to investigate Apple Replant Disease adopted (1). Trees were divided into two groups: those planted in a sterile loam and 'diseased' soil mix, and those planted in sterile loam as part of the control group. 'Diseased' soil and sterile loam was then amended with the novel green manure at three concentrations using fresh material (5g, 25g, 45g). The experimental period ran from 2012 until 2014, after which each tree were destructively analysed and soil samples underwent DNA analysis.

A subset of data for mean dry weight (g) of roots were statistically tested using Kruskal Wallis and pairwise comparisons completed thereafter.

RESULTS

Trees planted in diseased soil had roots that were significantly lighter in terms of mean dry weight (g) than those planted in sterile loam and those that had been amended with the three different concentrations of NGM ($p < 0.05$). Diseased soil amended with 45g of NGM resulted in a greater mean dry weight (g) than diseased soil amended with both 5g and 35g of NGM ($p = 0.021$ and $p = 0.004$, respectively). This was also demonstrated when compared to the 45g NGM amendment to sterile loam ($p = 0.001$) (Figure 1).

In the diseased soil SRD associated pathogens *Fusarium*, *Pythium*, and *Rhizoctonia* were all present. DNA analysis demonstrated that a 45g NGM amendment resulted in a reduction of pathogens in the soil and the removal of *Rhizoctonia solani*.

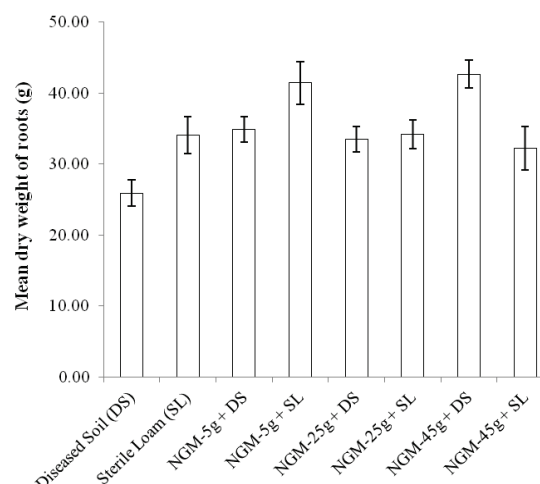


Figure 1. Effect of soil treatment on the mean dry weight of the roots of *S. aucuparia*. Controls used: Diseased Soil (DS) and Sterile Loam (SL). Application: Novel Green Manure (NGM).

DISCUSSION

Results from root dry weight (g) and DNA analysis suggest that the effect of the 45g NGM amendment on root growth is not a result of additional nutrients and are consistent with previous studies which demonstrated the control of *R. solani* and the reduction of the disease complex of three pathogens to two by NGM. Other research has indicated that suppression of this pathogen results in increased growth of apple seedlings (2).

This result lends itself to being transferrable to the treatment of replant disease in other rosaceous species. It also has the potential to open up a new avenue in the NGM is potentially an environmentally sensitive alternative to broad spectrum chemical fumigants.

ACKNOWLEDGEMENTS

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Pathogenicity determinants and high level of variation in the multinucleate genome of a single *Rhizoctonia solani* AG8 isolate

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INTRODUCTION

Rhizoctonia solani is a soil-borne basidiomycete fungus with a necrotrophic lifestyle which is classified into fourteen reproductively incompatible anastomosis groups (AGs). One of these, AG8, is a devastating pathogen causing bare patch of cereals, brassicas and legumes. *R. solani* is a multi-nucleate heterokaryon containing significant heterozygosity within a single cell (Figure 1).

MATERIALS AND METHODS

To gain insight into the biology of this multi-nucleate heterokaryon we sequenced the genome of a field isolate of *R. solani* AG8 without prior reduction of the genetic complexity. We assembled a haploid representation of the genome and used comparative genomics with other fungal pathogens of plants and non-pathogens to identify genes potentially associated with pathogenicity. Effector like genes were identified and proteomics used to complement in-silico predictions. Mutational analyses in *Arabidopsis* and *Medicago truncatula* were used to identify components of plant defences against *R. solani*.

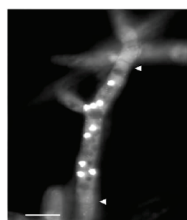


Figure 1. Fluorescence micrograph of *Rhizoctonia solani* AG8 hyphae (stained with SYBR green) displaying multiple nuclei within a single cell. Nuclei appear as brightly fluorescent structures. Hyphal septa are indicated with arrows and the scale bar is equivalent to 20 μ m.

RESULTS AND DISCUSSION

A high quality genome assembly of *R. solani* AG8 including 13,964 manually curated genes was produced and included RNAseq and proteomic support (1). We observed SNP-level 'hypermethylation' of CpG dinucleotides to TpG between nuclei, with similarities to repeat-induced point mutation (RIP). Unlike mononuclear fungi of the Pezizomycotina, gene-coding regions were widely affected along with repetitive DNA. *R. solani* was observed to contain higher levels of diversity within its' multiple nuclei to that held within sequenced populations of haploid fungal pathogens (Table 1).

Comparative analyses with 197 phytopathogenic taxa, known pathogenicity genes and other *R. solani* isolates predicted biological processes relevant to AG8 and 308 proteins with effector-like characteristics. Effector-like proteins had elevated ratios of non-synonymous to synonymous mutations (dN/dS), suggesting they may be under diversifying selection.

Table 1. SNP frequencies in genome comparisons.

	Comparison between	Multinucleate	Pathogen	SNPs/Kb
<i>R. solani</i> AG8 vs AG8	Nuclei (1-15)	Y	Y	14.0
<i>R. solani</i> AG8 vs AG1-IA	Anastomosis groups	Y	Y	176.0
<i>R. solani</i> AG8 vs AG1-IB	Anastomosis groups	Y	Y	153.6
<i>R. solani</i> AG8 vs AG3	Anastomosis groups	Y	Y	115.3
<i>Puccinia striiformis</i> (stripe rust) (2)	Nuclei (2)	Y	Y	6.0
<i>Blumeria graminis</i> (barley powdery mildew) (3)	Isolate pairs	N	Y	1.0
<i>Lentinula edodes</i> (shitake mushroom) (4)	Population	N	N	4.6
<i>Leptographium longiclavatum</i> (beetle symbiont) (5)	Population	N	N	0.6
<i>Rhizophagus irregularis</i> (endomycorrhiza) (6)	Multiple nuclei	Y	N	0.2

The moderate resistance to *R. solani* AG8 in *Medicago truncatula* is dependent on ethylene signalling which can be manipulated to give higher resistance (7). However, the strong resistance in *Arabidopsis* is independent of common defence signalling pathways but depends on reactive oxygen species production, both from the mitochondrial electron transport chain and RBOHs (8, 9). These findings suggest diverse pathways may be involved in responses to this broad host range pathogen in various plants.

ACKNOWLEDGEMENTS

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The impact of root diseases on subterranean clover pastures across Australia

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INTRODUCTION

Subterranean clover (*Trifolium subterraneum*) is the most widely sown annual pasture legume species in southern Australia, it is well adapted to our Mediterranean-type climate having been sown over an estimated area of 29 million ha (2). It forms the basis of many permanent or semi-permanent pastures in the medium to high rainfall areas, often in mixtures with grasses and other legumes. However, over the last two decades there have been widespread reports from producers of a general decline in their annual pastures in the livestock areas even in an excellent growing season.

Wool producers inherently face critical feed shortage across the autumn-winter months, coinciding with the main soil-borne pathogen attack and consequent damage from substantial pre- and post-emergence damping-off and seedling root disease in subterranean clover pastures. This can inflict losses of up to 40-45% in the most disease prone areas. Such losses consist of up to 30-90% of seedlings failing to emerge, 10-40% of surviving seedlings succumbing to post-emergence, and with a 35-70% reduction in growth of survivors (1). Further, root disease that establishes on seedlings (and winter productivity is a direct function of initial plant density) further develops to also affect mature clover plants and significantly reduce overall productivity of annual pasture legumes throughout the growing season

MATERIALS AND METHODS

A survey was undertaken to determine the extent of root disease on subterranean clovers across southern Australia in 2014. Farmer fields were sampled ($n=202$ with useable data) over autumn and winter of 2014 from across southern Australia, including Western Australia, South Australia, Victoria and New South Wales.

RESULTS AND DISCUSSION

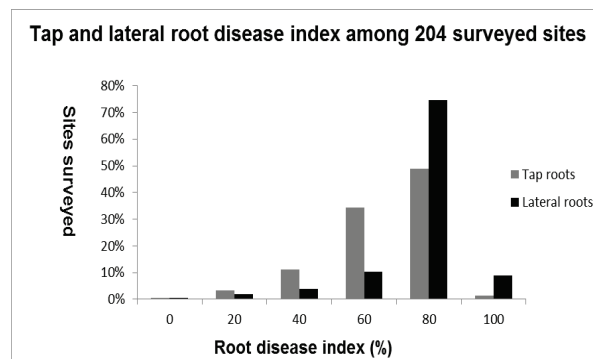
The vast majority of samples not only had a very severe level of tap root rot (with a tap root rot disease index of 60 to 80%), but that they also have extremely severe lateral root rot disease (with a disease index of 80 to 100%) (Fig. 1). It was evident that severe tap and lateral root disease occurred consistently across NSW, SA, Victoria and WA. Clearly, the combination of severe tap as well as lateral root disease (Fig. 1) is seriously aggravating the already challenging autumn-early winter feed gap for producers. However, the extent to which producers underestimated and under reported their levels of root disease in the survey, was startling; it is clear most producers were initially totally unaware of the very high levels of root disease in their pastures. It is of concern that root disease could be so severe across southern Australia with few producers (or consultants) having any real concept or idea of its occurrence let alone its severity. Though the feedback already provided to the majority of participating wool producers has addressed this issue. Nearly all participating producers now have heightened awareness of root disease challenges for sub clover pastures.

Tests for the presence of identifiable pathogens revealed that a range of major soil-borne pathogens was present widely across the samples, as would be expected in

association with such severe tap and lateral root disease. *Phytophthora clandestina*, *Aphanomyces trifolii* and *Pythium*, *Rhizoctonia* and *Fusarium* species were all widespread across southern Australia. Of these pathogens, *P. clandestina* is considered to be the most important. As expected, *P. clandestina* was widespread but not universally associated with all diseased samples.

This research has shown conclusively that subterranean clover pastures are severely impacted by root disease and is more widespread than previously understood. Many of the subterranean clover plants submitted were older varieties, (that producers could often not identify) which are more susceptible to root rot disease. Research staff and consultants should therefore encourage producers to re-establish their pastures with more recently released cultivars with improved root-rot resistance. In addition, the selection of subterranean clover for resistance to a range of common root-rot pathogens must continue to be a primary breeding objective. Further, it is noteworthy severe root disease is not limited in any way to be across only some specific rainfall zones; but rather is likely a serious problem and challenge across all zones.

Fig 1. Tap and lateral root disease incidence across southern Australian subterranean clover pastures in 2014.



ACKNOWLEDGEMENTS

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Investigating soilborne nectriaceous fungi associated with black root rot in avocado

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INTRODUCTION

Black root rot disease is considered to be a severe disease of young avocado trees and is associated with a complex of nectriaceous fungi including *Calonectria*, *Ilyonectria*, *Cylindrocladiella* and *Gliocladiopsis* spp. Root disease symptoms include black to brown-black necrotic lesions distributed throughout the roots which coalesce, producing rotten, black and reduced root population. Above-ground symptoms include stunted trees, leaf wilt and chlorosis, necrosis and browning, and leaf drop prior to leaf death. Nursery avocado seedlings with black root rot disease have been reported to die shortly after transplanting into orchards (within 1-5 years) causing significant commercial loss in Australia and in numerous countries including New Zealand, Colombia, Italy, Israel and Chile^[1]. This research aims to utilise molecular phylogenetic analysis of gene sequences to identify the nectriaceous fungi found in diseased avocado in Australia; and to perform pathogenicity tests on avocado seedlings and fruit to identify species pathogenic to avocado and other hosts.

MATERIALS AND METHODS

Species Identification Fungal isolates were obtained from symptomatic avocado roots from mature orchard trees, young orchard transplants, nursery stock, and from other host species from across Australia. DNA extraction, PCR amplification and sequencing of ITS, β -tubulin and histone 3 gene loci followed by sequence assembly, multiple alignments and maximum likelihood analysis generated phylogenetic trees for species identification and taxonomic classification.

Pathogenicity Tests *Calonectria ilicicola* (*Ca. ilicicola*), *Cylindrocladiella* spp. and *Gliocladiopsis* spp. isolates (from avocado), were inoculated on 12-week-old cv. Reed seedlings, by soil amended media containing a single isolate. Plant heights were recorded each week post inoculation for 5 weeks, then uprooted, the leaves and stems weighed and the roots weighed and assessed for severity of necrosis. This experiment was also performed with isolates obtained from other hosts. The isolates included *Ca. ilicicola* from papaya, peanut and custard apple, *Calonectria pauciramosa* from blueberry and *Ilyonectria liriodendri* from grapevine. Two trials were performed for each experiment. Culturing from necrotic root tissue was undertaken to satisfy Koch's postulates.

RESULTS AND DISCUSSION

More than 120 Nectriaceae isolates were collected with 30 isolates identified to date by gene sequencing, including 6 *Calonectria* sp., 15 *Ilyonectria* sp., 2 *Cylindrocladiella* sp. and 7 *Gliocladiopsis* sp., allowing the facilitation of classifying cryptic isolates as potentially new species. Glasshouse tests confirmed that two isolates of *Ca. ilicicola* were severely pathogenic, causing wilting or seedling death in 63% of seedlings 5 weeks after inoculation, whereas none of the mock, *Cylindrocladiella* or *Gliocladiopsis*-inoculated seedlings wilted or died in that time. Seedling height and leaf, stem and root biomass were also reduced significantly ($P < 0.001$) by *Ca. ilicicola*, while *Cylindrocladiella* and *Gliocladiopsis* were confirmed as non-pathogenic (Table 1).

Ca. ilicicola isolated from papaya, peanut and custard apple are pathogenic to avocado (data not shown), inferring a potential broad host range. Inoculation of fruit with *Ilyonectria* and *Ca. ilicicola* produced necrotic lesions in 100% of wound-inoculated sites. Further studies will include investigating the role of phytotoxins in pathogenicity, comparing susceptibility between rootstock varieties and evaluating potential disease management methods.

Table 1. Mean plant height (cm), root biomass (g) and percentage of necrotic roots (%) in avocado cv. Reed at 5 weeks post inoculation

Isolate	% root necrosis	Height (cm)	Root FW(g)	Root DW(g)
Uncolonised [*]	22.2 b	46.9 a	12.9 b	1.3 b
<i>Ca. ilicicola</i> [†]	78.1 a	38.1 b	3.1 c	0.4 c
<i>Ca. ilicicola</i>	70.6 a	41.4 a	2.9 c	0.5 c
<i>Cylindrocladiella</i>	30.0 b	45.2 a	16.3 a	1.7 a
<i>Gliocladiopsis</i>	34.0 b	48.2 a	14.8 b	1.6 b
<i>Gliocladiopsis</i>	24.9 b	44.5 a	14.1 b	1.5 b

Mean values within columns with the same letter are not significantly different at $P < 0.05$



Figure 1. At 5 weeks post inoculation: death (A), wilt and stunting (A & B) caused by *Ca. ilicicola* compared to uninoculated control plants (C).

ACKNOWLEDGEMENTS

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Characterisation of the blueberry stem blight pathogen in northern NSW

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INTRODUCTION

A range of *Botryosphaeriaceae*, including *Neofusicoccum* and *Lasiodiplodia*, cause stem blight in horticultural crops, including the blueberry industry in Northern NSW. The species of *Botryosphaeriaceae* involved are not well understood, preventing the development of targeted management strategies. This project aims to characterise the *Botryosphaeriaceae* species associated with blueberry stem blight. Fungi isolated from asymptomatic and symptomatic tissue will be identified using morphological and molecular techniques. The pathogenicity of these isolates will be assessed using a attached and detached twig assays to determine the species responsible for the disease in Northern NSW. This project will improve our understanding of an important disease in a high value and rapidly growing industry.

MATERIALS AND METHODS

Samples were collected from a commercial blueberry (*Vaccinium corymbosum* × *V. darrowi*) orchard in Corindi, NSW. *Botryosphaeria* isolates from symptomatic blueberry tissue were also obtained from Gingin, WA. Tissue isolations were made from surface sterilised (0.5% NaOCl, 60 s) sections of both infected and uninfected stems onto 1/4 strength potato dextrose agar (PDA). Single hyphal tips were taken to obtain cultures on ¼ PDA.

DNA was extracted using the Fast prep DNA extraction protocol. MyTaq DNA polymerase (Bioline Pty Ltd Alexandria, NSW, Australia) was used to amplify the internal transcribed spacer (ITS) and elongation factor 1- α (EF1- α) gene regions. ITS and EF1- α were sequenced using primers ITS 1 and ITS 4 and EF1-688F and EF1-1251R respectively.

Spore suspensions for inoculations were prepared by transferring sections of 7-10 day old cultures onto 2% water agar with autoclaved pine needles. These were incubated under 12 hour day/night cycles until sporulation was observed. Mature conidiomata from each *Botryosphaeriaceae* isolate were crushed in 1.5 mL sterile microcentrifuge tubes to release conidia, then filtered to remove mycelial and pycnidial fragments. Spore concentrations were adjusted to a final density of 1×10^5 conidia/mL. Inoculum (15 μ L) was applied to incisions on the stem. Inoculation points were bound with parafilm to prevent desiccation. Lesion development and colonisation was assessed for 4-6 weeks.

RESULTS

A maximum parsimony analysis was undertaken using the ITS and EF- α 1 loci to compare the preliminary isolates. Preliminary analysis suggests a range of *Neofusicoccum* species are present in blueberry in northern NSW, including *N. macroclavatum*, *N. occulatum* and *N. parvum*. *Neofusicoccum parvum* was the most frequently isolated species from diseased and healthy blueberry stem tissue. Additionally, *Lasiodiplodia theobromae* has also been isolated from both diseased and healthy blueberry stem tissue. Pathogenicity testing is in progress and will be reported.

DISCUSSION

This study demonstrates that a number of members of the *Botryosphaeriaceae* may be involved in stem blight in blueberry. Further phylogenetic analysis will give a greater understanding of the diversity of species involved.

Pathogenicity testing will determine which of the isolated species are associated with stem blight on blueberry. Some of the species isolated are known to be pathogenic organisms in other host plants. *Lasiodiplodia theobromae* is a known agent of blueberry stem blight in the North American blueberry industry (2). As it was found in both diseased and asymptomatic tissue, it is clear that this species can exist as both an endophyte and pathogen depending on environmental conditions.

N. parvum is also a known pathogen. It has been associated with stem canker of grapes and stem blight of blueberries in New Zealand (3). *N. occulatum* is also known to cause dieback in grape crops internationally (4). A knowledge of specific species involved with stem blight allows for the utilisation of specific knowledge of their disease cycle in relevant systems in order to synthesise more targeted management plans.

ACKNOWLEDGEMENTS

We wish to thank Costas—Berry Category for access to the sample material utilised in this study.

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Investigating the pathogenicity of *Neocosmospora* species on germinating chickpea seedlings under controlled conditions

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INTRODUCTION

Several species belonging in the genus *Neocosmospora* including *N. vasinfesta* var. *africana* and *N. striata* have been reported as pathogens of peanut (*Arachis hypogea*) (1), chickpea (*Cicer arietinum*), soybean (*Glycine max*) and other plants in Australia and overseas.

Consistent disease symptoms have been observed overseas for all three crop species, with leaf chlorosis and wilting of plants either scattered or in groups across affected fields (2,3). The *Neocosmospora* species cause extensive damage to the root systems of all three hosts and often produce orange-red spherical perithecia on infected decaying roots or stem lesions. Stem lesions extending 10-15cm (2) upwards from the soil surface often develop on infected soybean plants.

Neocosmospora root rot (causal species yet to be fully determined) is prevalent through most peanut growing regions in Australia. Yield losses from disease outbreaks have been estimated to be between 30-100% with an annual economic value of up to \$5.4 million. No yield or economic loss data are available for chickpea or soybean either in Australia or worldwide.

MATERIALS AND METHODS

Chickpea seed of the commercial variety PBA HatTrick was surface sterilised for 30 minutes in a 4% sodium hypochlorite solution and rinsed five times with deionised water. Seed was sown on 2% water agar plates and incubated for seven days. Millet seed inoculated with a mixture of two isolates of *Neocosmospora* sp. (BRIP56976, BRIP56979) collected from field affected peanut plants was used as inoculum.

Treatment A (A) consisted of a 10mm layer of millet seed that was placed in the bottom of sterile plastic 30mL containers and covered with sterile vermiculite. Treatment B (B) comprised inoculated millet mixed through sterile vermiculite and treatment C (C) had inoculated millet placed on top of the vermiculite. Sterile millet seed was used for the control treatment (D). A clean, germinated chickpea seed was transplanted from the water agar plates onto the vermiculite into each tube and watered from above.

Ten replications of each treatment were then placed into a controlled environment cabinet at 23°C on a 16h photoperiod cycle and watered every two days using a fine spray from above.

RESULTS

All three inoculation methods resulted in seedling death resulting from colonisation of the germinated seed before and after coleoptile emergence.

Every seed in treatment A was rapidly colonised by the *Neocosmospora* sp., which prevented coleoptile emergence. Treatments B and C were significantly less

effective methods of inoculation than Treatment A with no significant difference in seedling mortality between them ($P>0.05$) after 21 days (Figure 1). Seedling death was more rapid in treatment B than in treatment C (Table 1) however no significant difference could be reported between treatment C and the control ($P>0.05$). The seedling deaths observed in treatment D (control) were not due to *Neocosmospora* infection.

% Seedling Mortality

Figure 1. The percentage of seedling death at 21 days after inoculation caused by the *Neocosmospora* sp. for treatments A, B, C and D.

Table 1. Seedling mortality (expressed as %) at 7, 14 and 21DAI.

Treatment	7DAI	14DAI	21DAI
A ^A	100	100	100
B ^B	27	36	36
C ^{B,C}	0	15	50
D ^C	0	0	10

DISCUSSION

The results of this experiment indicate that treatments B and C are effective in causing infection of chickpea seedlings by *Neocosmospora* sp. without killing the seed before full emergence is achieved. This technique could be used to screen Australian chickpea varieties for their relative resistance, as well as to investigate the pathogenicity of *Neocosmospora* sp. on other legume crops.

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The impact of root-lesion nematodes, *Pratylenchus* spp., on summer grain crops

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INTRODUCTION

Summer grain crops grown in the northern grain region of Australia can play a pivotal role in the management of the root-lesion nematodes, *Pratylenchus thornei* and *P. neglectus* because each nematode species has a broad, but differing host range. These nematodes are commonly found throughout the region and cause up to 50% yield loss in wheat and up to 20% in chickpea. Management relies on growing crops with tolerance, so that crop yield is maximised, and rotating with resistant crops to decrease damaging nematode populations. Here we report new data on the tolerance of mungbeans and resistance of current cultivars and advanced lines of sorghum and maize.

MATERIALS AND METHODS

Glasshouse Sorghum and maize cultivars were grown in pots containing pasteurised vertosol soil for 19 weeks on a capillary-action bottom-watering system. Each pot was inoculated at planting with 10,000 *P. thornei* or *P. neglectus*/kg soil. A set of cereal cultivars with known responses to *P. thornei* or *P. neglectus* (standards* in Table 1) was included in each experiment; treatments were replicated 3 times. Nematodes were extracted from roots and soil by the Whitehead tray method for 48h and then counted under the microscope in a 1-mL Chalex slide. Reproduction factor (RF) was calculated as the final nematode population divided by the initial population.

Field Two field experiments were conducted at Formartin on the eastern Darling Downs, Queensland (24.464°S, 151.426°E). In Year 1, plots with high or low populations of *P. thornei* were prepared by planting susceptible wheat cv. Kennedy, or partially-resistant wheat cv. QT8343 in a row:column design. In Year 2, 10 cultivars of mungbean and a susceptible soybean cv. were planted into the prepared plots following a 1-month or 13-month fallow after the Year 1 wheat; an unplanted treatment was included in Year 2. Each plot was sampled in Year 2 to 90 cm soil depth to determine nematode populations at planting and at harvest.

Statistical analysis Nematode data were transformed by $\ln(x+1)$; all data were analysed by REML in Genstat.

RESULTS AND DISCUSSION

Sorghum and maize Current commercial sorghum hybrids were susceptible to *P. neglectus* (RF 2.1–8.8); for other genotypes, RF ranged from 0.9 for *Sorghum arundinaceum* to 15 for the susceptible sorghum control cv. MR Goldrush. The RF for *P. thornei* on maize cultivars ranged from resistant to susceptible (RF 0.7–6.0) (Table 1). There is clearly a need for further screening of current cultivars before release, particularly for maize where there are a range of responses to *P. thornei*. Development of resistant cultivars of both sorghum and maize is needed.

Mungbean There was a 5-fold difference in *P. thornei* populations following the Year 1 wheat treatments. Grain yield of the mungbean cultivars in Year 2 was reduced by 5–10% for some cultivars. At the time of writing, analysis of nematode population data was not completed.

Table 1. Examples of the range of responses 19-weeks after inoculation of sorghum cultivars with *Pratylenchus neglectus* or maize with *P. thornei*. RF; reproduction factor. *, cereal standards.

Sorghum	<i>P. neglectus</i> /kg soil	
	$\ln(x+1)$	RF
Unplanted	7.28	0.1
Abacus*	8.12	0.3
<i>Sorghum arundinaceum</i>	9.13	0.9
SC170-6-8	9.41	1.2
MR-Buster	10.36	3.2
HGS-102	10.50	3.6
Pacific MR43	10.58	3.9
HGS-114	10.60	4.0
MR-Eclipse	10.72	4.5
S4	10.86	5.2
84G99	10.95	5.7
Dominator	11.28	7.9
MR-Bazley	11.38	8.8
IS8525	11.40	8.9
84G22	11.47	9.6
S9	11.60	10.9
MR Goldrush	11.91	14.9
Brookton*	11.99	16.1
<i>l.s.d.</i> ($P=0.05$)	1.17	

Maize	<i>P. thornei</i> /kg soil	
	$\ln(x+1)$	RF
GS50a*	8.14	0.3
HM-114	8.26	0.4
PAC301	8.48	0.5
unplanted	8.62	0.6
PAC735	9.27	1.1
HM-102	9.47	1.3
NEW PAC606	9.63	1.5
P1419E	9.67	1.6
33V62	9.90	2.0
NEW PAC607IT	9.92	2.0
HM-113	10.03	2.3
PAC727	10.20	2.7
PAC345IT	10.25	2.8
HM-101	10.28	2.9
DK764	10.41	3.3
PAC624	10.75	4.6
Batavia*	11.27	7.9
<i>l.s.d.</i> ($P=0.05$)	1.41	

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Plant root knot nematode interaction: a sophisticated dialogue

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The Root Knot Nematode (RKN) *Meloidogyne incognita* is a widespread and polyphagous obligate asexual endoparasite of plants that causes serious and growing problems to agriculture. This lifestyle implies dramatical changes of plant cells into specialised feeding sites, which are induced by secreted proteins by the nematode, so-called parasitism effectors. An integrated approach of molecular techniques has been used to functionally characterise nematode parasitism proteins. The complete genome sequence of *M. incognita* revealed that the assembled sequence consists of homologous but divergent segment pairs that might represent former alleles in this species. Based on comparative genomics, we identified in RKN genomes a set of genes preserved during the evolution and of plant-parasitic nematodes only shared with organisms having a plant parasitic lifestyle. Using a series of bioinformatic screens, we selected a set of genes that were further analysed with design of siRNAs and infestation test experiments after silencing. Inactivated genes showed a significant reduction in the number of egg masses or gall numbers compared to the control. These genes represent new targets allowing to develop new methods to control plant-parasitic nematodes but harmless for the environment and the consumer health. Thus, RKNs constitute a unique model system to study the links between variation in genome structure, mode of reproduction, and adaptation to environment and hosts, in relation with parasitic success.

Cercosporoid tree of life

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INTRODUCTION

Cercosporoid fungi represent one of the largest groups of hyphomycetes belonging to the *Mycosphaerellaceae* (Ascomycota) (1). Most species are leaf-spotting plant pathogens and several cause serious damage to important crops worldwide. Species in this group are mostly asexual with a relation to mycosphaerella-like sexual morphs. The sexual morph is morphologically rather uniform in this complex whereas the asexual morphs are quite diverse. Cercosporoid fungi are defined as chiefly dematiaceous hyphomycetes with conidiophores formed singly or in groups, having intercalary, integrated or terminal conidiogenous cells, and holoblastic conidiogenesis forming amero to scolecosporous conidia, solitary or in chains (2).

These fungi represent a very heterogenous group for which the only existing monograph (3) is in urgent need of revision. With the introduction of phylogenetic analysis based on DNA sequences the *Mycosphaerellaceae* has been more narrowly defined with names of asexual genera being used to identify morphologically distinct monophyletic clades (e.g. *Cercospora*, *Pseudocercospora*). However, several genera appear to be polyphyletic showing that some morphological characters have evolved more than once within the family (e.g. *Passalora* and *Zasmidium*). Several valid cercosporoid genera have an uncertain status since no ex-type culture is available (e.g. *Distocercospora*). In this study we compared materials of 19 genera: *Asperisporium*, *Cercospora*, *Cladosporiella*, *Denticularia*, *Distocercospora*, *Elletevera*, *Eriocercospora*, *Eriocercospora*, *Pallidocercospora*, *Pantospora*, *Parastenella*, *Passalora*, *Pseudoasperisporium*, *Pseudocercospora*, *Pseudocercosporidium*, *Quasiphloeospora*, *Scolecoglyphina*, *Semipseudocercospora*, *Sirosporium*, *Stenella* and *Zasmidium*.

Understanding and stabilising the taxonomy of cercosporoid fungi is urgent, given the impact of these species in agriculture and forestry. This is a challenging task since species descriptions are scattered throughout the literature, and only a few strains are known from culture. In this study we compiled a multigene phylogenetic analysis of several cercosporoid genera that had ex-type strains available for study.

MATERIALS AND METHODS

The 350 isolates included in this study were obtained from the CBS and CPC culture collections, both housed at the CBS-KNAW Fungal Biodiversity Centre, the Netherlands. DNA was isolated using the UltraClean™ Microbial DNA Isolation Kit. Three partial nuclear genes were amplified and sequenced: 28S nrRNA gene (LSU), internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS) of the nrRNA operon and RNA polymerase II second largest subunit (RPB2). New primers were designed for the amplification of RPB2. Multiple sequence alignments were constructed in MAFFT. Phylogenetic trees of concatenated gene alignments were constructed by means of Bayesian analysis (Mr.Bayes v. 3.2.1), Maximum likelihood (RAxML)

and Parsimony (PAUP v. 4.0b10). Morphological characterisation of new species was performed using a Nikon Eclipse 80i light microscope with differential interference contrast (DIC) illumination and using lactic acid as mounting medium.

RESULTS AND DISCUSSION

The dataset includes strains from 40 genera belonging to the *Mycosphaerellaceae*, two belonging to the *Teratosphaeriaceae* and one to the *Schizothyriaceae*.

From the 19 genera studied only 11 were supported with cultures: *Asperisporium*, *Cercospora*, *Distocercospora*, *Pallidocercospora*, *Pantospora*, *Passalora*, *Pseudocercospora*, *Scolecoglyphina*, *Sirosporium*, *Stenella* and *Zasmidium*.

The type of *Stenella* was found to cluster in the *Teratosphaeriaceae* and the *Stenella* strains remaining in the *Mycosphaerellaceae* were consigned to *Zasmidium*, with several novel species being introduced. The type species of *Asperisporium* was found to cluster close to *Amycosphaerella*. The type of *Pantospora* (*P. guazumae*) clustered close to the type of *Pseudocercospora* (*P. bakeri*). Likewise the type of *Scolecoglyphina* (*S. mangiferae*) clustered close to *Pallidocercospora* (*P. heimi*). Presently no ex-type strains are available for *Sirosporium*, and the two species used in this study clustered in different clades. *Passalora* was revealed to be paraphyletic, leading to the introduction of several novel genera. *Distocercospora* proved to be allied to *Pseudocercospora*, but distinct from *Cercospora*.

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Unraveling the taxonomy of the *Colletotrichum* species causing anthracnose in chili in Australia and SE Asia

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INTRODUCTION

Colletotrichum is one of the most frequently reported groups of plant pathogenic fungi causing anthracnose disease in crop plants throughout the world. Apart from *C. truncatum*, the species with oblong conidia, *C. acutatum* and *C. gloeosporioides*, have been shown to be the two major species complexes causing chili anthracnose (1). Recently the *Colletotrichum* spp. belonging to the *C. acutatum* complex infecting *Capsicum* were identified as *C. scovillei* in SE Asia and *C. brisbanense* in Australia, and species of the *C. gloeosporioides* complex were identified as *C. siamense* in SE Asia (1). Understanding the taxonomy and pathogenicity of these species complexes has great significance to the Australian chili industry where there are serious biosecurity implications from incursion by new exotic pathogens entering from SE Asia. In previous studies species within each *Colletotrichum* complex have been identified using multigene phylogenetics employing sequences of five to seven genes (1). Furthermore, the ApMat gene-marker was specifically designed to delineate the *C. gloeosporioides* species complex (2), and has subsequently been used in molecular phylogenetic analyses (4). The aim of the present study was to determine the taxonomy and phylogeny of *Colletotrichum* species from countries in SE Asia and Australia using multigene phylogeny.

MATERIALS AND METHODS

A collection of 25 *Colletotrichum* isolates with oblong conidia from Australia, Thailand, Malaysia and Sri Lanka was grown in potato dextrose agar (PDA) and incubated for 5-7 days at 27°C. The *Colletotrichum* isolates were primarily identified based on culture characteristics on PDA, conidium morphology and ITS sequence data. All isolates were analysed using various combinations of genes including the internal transcribed spacer (ITS) region, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), chitin synthase 1 (CHS-1), actin (ACT), glutamine synthetase (GS), partial mating type gene (ApMat) and beta-tubulin (TUB2). Isolates were sequenced using the primer pairs ITS-1F + ITS-4, GDF1 + GDR1, CHS-354R + CHS-79F, ACT- 512F + ACT-783R, AMF1 + AMR1, + GSF1 + GSR1 and BT2Fd + BT4R respectively (2). Multiple sequence alignments were constructed in MEGA v. 6.06. Phylogenetic trees based on concatenated genes were constructed in Bayesian analyses performed using MrBayes v. 3.2.2

RESULTS AND DISCUSSION

***Colletotrichum acutatum* complex.** The combined five gene analysis of ITS, GAPDH, CHS-1, ACT and TUB2 on the selected 10 isolates that belong to the *Colletotrichum acutatum* complex enabled a better resolution of species with nine isolates being closely associated with *C. scovillei*. One isolate formed a separate clade which was distantly related to the *C. boninense* complex. These results correlated with the findings from a previous study (2), in which the taxonomy of the *C. acutatum* complex was

revised, and the pathogen of chili named as *C. scovillei* based on isolates from Thailand and Indonesia.

***Colletotrichum gloeosporioides* species complex.** The phylogenetic analysis of the *C. gloeosporioides* species complex using the ApMat and GS gene loci showed that all the isolates clearly separated into three sub-clades representing three different geographical locations. Mainly three isolates from Australia which formed a small sub-group and seven isolates from Thailand showed close relation to the epitypes *C. siamense* and *C. queenslandicum*, which were significantly different from the Sri Lankan isolates and one Thai isolate which clustered separately from others. Comparatively, combined gene analysis of ITS and TUB2 data were not informative enough to separate the above isolates. However, phylogenetic trees built from ApMat and GS genes provided similar information and support as the concatenated tree of four genes (ApMat, ITS, TUB2, GS). This study demonstrated that the ApMat marker was an informative marker that distinguished most species in the *C. gloeosporioides* species complex, and provided high resolution at species level. Identification of potentially new *Colletotrichum* species and further characterisation of their morphology has to be completed using more isolates and multi-gene sequence data.

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Characterisation of *Diaporthe* species complex on soybean

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INTRODUCTION

The genus *Diaporthe* comprises large number of cosmopolitan plant pathogens on wide range of hosts. Four *Diaporthe* taxa are described as soybean pathogens: *D. sojae*, causal agent of pod and stem blight; *D. caulivora* and *D. aspalathi*, agents of northern and southern stem cankers, respectively; and *D. longicolla*, the most common and most damaging agent of soybean seed decay (1). Taxonomy of *Diaporthe* species was subject of many studies for a long time. Currently, these species are redefined mostly based on a combination of morphological, cultural, mating type and DNA sequence data (1, 2, 3) with rarely examined pathogenicity. The objectives of this study were to identify *Diaporthe* species on soybean in Serbia and to consider their taxonomic status in terms of molecular, morphological and pathogenic data.

MATERIALS AND METHODS

Isolation and morphology Using standard methods, 160 *Diaporthe* strains were isolated from symptomatic plants and seeds collected throughout the soybean-producing area in Vojvodina Province, Serbia. Study included four *D. aspalathi* isolates from the USA. A total of 171 isolates represented different morphological groups recognised on potato-dextrose agar (PDA).

Sequence Analysis After DNA extraction, PCR amplification and sequencing of internal transcribed spacer (ITS1-5.8S-ITS2) region of rDNA, partial translation elongation factor 1 alpha (EF-1 alpha) and partial large ribosomal subunit (LSU) was performed (4). BLAST searches were carried out in order to select closely related sequences from GenBank. Phylogenetic trees were inferred in PAUP* 4.0b10.

Pathogenicity tests Pathogenicity was tested on plants and seeds of soybean cultivar Balkan, using mycelia and conidial suspensions (4). Five plants per pot were inoculated at V2 growth stages by plug method, while seeds were inoculated using conidial suspensions. Seeds were placed on wet filter paper in 90 mm Petri dishes with 25 seeds per dish, and incubated at 24°C in dark. The experiment was set up in four replications. After 7 days, number of germinated and decayed seeds was counted.

RESULTS

Identification Isolates were divided into four different morphological groups identified as four taxa: 51 isolates of *D. caulivora*, four isolates of *D. aspalathi*, 70 isolates of *D. longicolla*, and 46 isolates of *D. sojae*. BLAST analyses confirmed morphological identification of *D. caulivora* and *D. aspalathi*. From the third morphological group, 65 isolates were identified as *D. longicolla* and five as *D. novem*. The greatest variability was observed in *D. sojae*. Molecular identification showed that only 20 isolates belong to this taxon, while the rest 26 were identified as *D. eres*, *D. neotheicola* and *D. viticola*. Phylogenetic analyses showed clearly grouping of all identified species and defined their synonyms.

Pathogenicity The inoculation of soybean plants at V2 growth stages by inserting the mycelium plugs in wounds

on the stem showed that isolates of *D. longicolla*, *D. novem*, *D. aspalathi*, *D. caulivora* and *D. neotheicola* were highly pathogenic causing wilting of all plants, followed by 80% of wilting plants that were inoculated with *D. sojae* and 40% inoculated with *D. eres*. Dynamics of wilting plants was uneven. *D. viticola* did not cause wilting of plants. None of the control plants showed any disease symptoms. Inoculation of soybean seeds by immersing them into conidial suspensions showed that *D. longicolla*, *D. novem*, *D. sojae*, *D. aspalathi*, *D. caulivora* and *D. neotheicola* significantly reduced the germination rate of the seeds. These species caused seed decay with 100%, followed by 72% of seeds inoculated with *D. eres*, and 62% with *D. viticola*.

DISCUSSION

Accurate identification of pathogens and precise naming are essential for all aspects of phytopathology. The name *Diaporthe phaseolorum* has been conventionally used for *Diaporthe* species from soybean. From the moment of discovery until now, this species has changed several times the name or taxonomic category. Therefore, different names or taxonomic rank for same species is often used in literature, which is rather confusing and incomprehensible. One of the main reasons lay in fact that when scientists detect for the first time presence of already described *Diaporthe* species on new host plant, they declare it as new species and automatically assign it with brand new name. The greatest taxonomic confusion is linked up to *D. sojae*, because this variety is known for great variability of morphological characters. This study showed that it is not due to variability, but because of presence of other species. Several authors concluded that *D. sojae* and *D. longicolla* are very close related and are the same species (2). Using morphology, phylogenesis and pathogenicity, *D. longicolla* can be distinguished from *D. sojae*. On the other hand, *D. caulivora* and *D. aspalathi* are treated as separate species (1, 3). In this study, these two species are found to be phylogenetically close and should be treated as varieties because no fundamental differences in terms of molecular, morphological and pathogenic data were noted.

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Colletotrichum species associated with citrus anthracnose in Tunisia

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INTRODUCTION

Species belonging to the genus *Colletotrichum* have been recorded on a wide range of plants worldwide, including important crops. Identification of *Colletotrichum* species based on morphological features is difficult and confusing. However, molecular identification based on fungal gene sequences has resolved many new species. Single gene identification is not efficient to differentiate species and thus reliable taxonomy is now based on multigene phylogenetic analysis. The choice of the best combination of gene sequences to be used for a better resolution is still controversial, in particular for resolving species within the *C. gloeosporioides* species complex (4). Several *Colletotrichum* species have been reported to be associated with *Citrus* plants as pre-harvest and post-harvest pathogens as well as saprobes and endophytes (1, 3). Previous studies reported the significance of *C. gloeosporioides* (Penz.) Penz. & Sacc. in the cause of citrus anthracnose (3). *C. gloeosporioides* was originally described from *Citrus sienensis* in Italy (2) and some studies revealed it to be the most common species associated with diseased leaves and wither tip on twigs and tear stain of citrus (1, 3). Citrus anthracnose has been reported in many areas around the world and in particular in the Mediterranean basin, but has never been reported in Tunisia.

MATERIALS AND METHODS

Twenty-one isolates of *Colletotrichum* sp. were obtained from stems of different *Citrus* varieties (*Citrus sinensis*, *C. reticulata*, *Citrus x tangelo*, *C. clementina*), showing anthracnose-like symptoms in Tunisia and pre-selected from a wider collection of isolates based on morphological features. Four Australian *Colletotrichum* isolates obtained from *C. sinensis*, *C. reticulata*, *C. aurantiifolia* and *C. australasica* were included in this study for comparison.

Multilocus phylogenetic analyses were performed using four molecular markers: internal transcribed spacer (ITS), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), glutamine synthetase (GS) and ApMat sequences. Representative isolates were characterised based on cultural aspect, *in vitro* growth and microscopic features including spore and appressoria measurements and shape. In order to assess the ability of the major identified species to cause infection, pathogenicity tests were performed with representative isolates by inoculating wounded and non-wounded fruits and detached leaves of different citrus varieties.

RESULTS AND DISCUSSION

Based on the blast results from the NCBI database, all Tunisian isolates were preliminarily allocated to the *C. gloeosporioides* species complex. Multilocus phylogenetic analyses showed two main clades of isolates within the complex, with 19 and 2 isolates corresponding respectively to the species *C. gloeosporioides* and a putative new species. Among the isolates belonging to the first clade, two main sub-clades were observed, with 7 isolates clustered with the reference isolate of *C. gloeosporioides* (CBS112999) and 12 isolates that were slightly distant. One of the isolates in the first sub-clade showed high closeness

with the Australian *C. gloeosporioides* isolate from *C. sinensis*. The first subclade included isolates from *C. sinensis*, *C. reticulata* and *Citrus x tangelo*, whereas the second sub-clade comprised mainly isolates from *C. sinensis* and included two isolate from *Citrus x tangelo* and one isolate from *C. clementina*. The two isolates corresponding to the new species were both obtained from Encore Mandarin (*C. reticulata* Blanco). Specific morphological features of two main groups of isolates identified as *C. gloeosporioides* as well as those of the new species were determined. Pathogenicity tests confirmed the three groups of isolates to be pathogenic and revealed a significant (pathogen x host) interaction and an eventual specificity in causing infection after wounding or without wounding according to the inoculated varieties. Although our results are in agreement with previous studies that found that *Colletotrichum gloeosporioides* was the predominant pathogen of cultivated citrus species (3) there is also one new potential *Colletotrichum* species that causes anthracnose of citrus.

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Molecular phylogenetic analysis of Australian *Bipolaris* isolates reveals novel taxa and host records

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The helminthosporioid genus *Bipolaris* includes many important pathogens of grasses (Poaceae). These fungi are associated with diseases on more than 60 host plant genera. Until recently, the classification of *Bipolaris* spp. has been based entirely on morphological characteristics, especially of conidia and conidiophores. As *Bipolaris* and related genera (*Curvularia*, *Exserohilum*, *Johnalcornia*, *Porocercospora*) have similar morphology, this approach is unreliable for the allocation of new species. In the last decade, molecular phylogenetic approaches have provided additional reliable criteria that have allowed the development of a more stable taxonomy. A multilocus phylogenetic analysis (ITS, GAPDH, LSU, and EF-1 α) has provided clear generic boundary between *Bipolaris* and its closest relative, *Curvularia*, leading to the transfer of several species from *Bipolaris*. The same molecular phylogenetic method was applied to 20 isolates from the Queensland Plant Pathology Herbarium (BRIP) culture collection, revealing novel taxa and new host records.

Lasiodiplodia (Botryosphaeriaceae) associated with Myrtales from Southern Africa

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INTRODUCTION

Myrtales is an important order of plants in Africa and globally. *Eucalyptus* is the most economically significant genus with varying uses including pulp, paper and timber. Native forests are a reservoir of plant pathogens that may infect adjacent horticultural and forestry species and vice-versa. The fungal order Botryosphaerales contains devastating plant pathogens including *Diplodia sapinea*, and species of *Botryosphaeria*, *Dothiorella*, *Lasiodiplodia* and *Neofusicoccum* (1). Several species are latent pathogens that cause disease when the host is under stress (2).

Lasiodiplodia spp. (order Botryosphaerales) are cosmopolitan in distribution, occurring on most continents in tropical and sub-tropical climates, and are particularly associated with woody hosts. They cause fruit rots, stem-end rots, cankers, dieback, decline & tree death of crops including *Eucalyptus* spp. (3) and guava (4).

The aim of this study was to identify species of *Lasiodiplodia* associated with Myrtales in southern Africa, and describe any new species that could pose a potential disease threat to native and introduced Myrtales.

MATERIALS AND METHODS

Twigs with or without symptoms were collected from Myrtales hosts, mainly Combretaceae, Myrtaceae and Melastomataceae. The countries sampled included Botswana, Namibia and Zambia, with the majority collected in Zambia. The consolidated species concept was used to delineate species boundaries (5).

RESULTS AND DISCUSSION

Lasiodiplodia pseudotheobromae had the widest geographic distribution and host range. It was found on 10 different host species from *Combretum* (six species) (Combretaceae), *Terminalia* spp. (three species) (Combretaceae) and *Syzygium cordatum* (Myrtaceae).

Lasiodiplodia pseudotheobromae is a known pathogen of Myrtales including *Eucalyptus* (3, 7), *Terminalia catappa* (6) and *Terminalia sericea* (Begoude et al. 2009 unpubl.). In addition, *L. cryptotheobromae* was isolated from *Terminalia stuhlmanii* (Combretaceae), *Combretum arbutatum* (Combretaceae) and *Syzygium guineense* (Myrtaceae). This species is a known pathogen of *Terminalia sericea* (Combretaceae) (Begoude et al. 2009 unpubl.).

Two new species were also discovered, *L. combretae* prov. nom., and *L. zambeziaca* prov. nom. *L. combretae* was only found on species of *Combretum* indicating the host may have an effect on its distribution. *Lasiodiplodia zambeziaca* was found on *Combretum* (two species), *Terminalia* (two species) (Combretaceae) and *Dissotis phaeotricha* (Melastomataceae).

Evidence of hybridisation was found with some isolates grouping with different species, depending on the locus observed. Morphologically, single necks on conidiomata were observed with *L. zambeziaca*. Neckes have only been reported previously in this genus on *L. mahajangana*. Both

single and multiple necks were observed on *L. combretae*. This is the first record of multiple necks on a species of *Lasiodiplodia*.

The pathogenicity of the new species are currently unknown. tests Pathoogenicity tests need to be completed to determine the pathogenicity of the new species.

The findings of this research will equip plant pathologists in southern Africa with knowledge of emerging disease threats, increase the effectiveness of biosecurity & quarantine, and help inform government and policy makers.

ACKNOWLEDGEMENTS

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Phylogenetic relationships and population diversity of commonly found phytoplasma strains in Sri Lanka

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INTRODUCTION

Phytoplasmas are responsible for several hundred plant diseases, affecting many economically important crops globally, the devastating consequences, i.e. economic and social impacts, of which are often underestimated in developing countries.

In Sri Lanka several phytoplasma strains were identified of which coconut and sugarcane were the most affected plantation crops; Weligama coconut leaf wilt disease (WCLWD) is a critical threat to the coconut cultivation while sugarcane white leaf and grassy shoot diseases (SCWLD and SCGSD) reduce yield of sugarcane. Arecanut yellow leaf disease (AYLD) was recently reported in Sri Lanka. Bermuda grass white leaf disease (BGWLD) has been suggested as phytoplasma reservoirs. Therefore this study was undertaken to determine the phylogenetic relationships of the above phytoplasma strains and to evaluate the prevailing genetic and population diversity in Sri Lanka.

MATERIALS AND METHODS

Partial *secA* sequences of WCLWD, SCWLD, SCGSD, AYLD and BGWLD were generated by sequencing the amplicons primed by SecAfor1/SecArev3 and RicesecAfor2/RicesecArev3. Comparative phylogenetic analyses were performed using both maximum likelihood (ML) and Bayesian inference (BI) methods, of *secA* sequences from phytoplasma across 14 distinct 16Sr groups. Furthermore, each *secA* sequence was subjected to *in silico* digestion with 22 restriction enzymes available in pDRAW32 software followed by virtual agarose gel electrophoresis. The similarity coefficient (F) was calculated (1).

Population diversity parameters i.e. DNA polymorphism, nucleotide diversity (π), average nucleotide differences (k) and haplotype diversity (Hd) were determined by DnaSP version 5.10.01. The expectation of the neutrality of the population was determined by the Tajima's D test and the intra and inter population genetic diversity was calculated, where the distance between the populations was measured by the Fixation Index (F_{ST}).

RESULTS

Both *secA* based ML and BI analyses remained consistent in grouping, where WCLWD and AYLD phytoplasmas were clustered together with BGWLD phytoplasma of the 16SrXIV group, evidencing a nonconformity with the 16S rDNA based existing classification where WCLWD was classified under 16SrXI (2). Importantly, the phylogenetic position of AYLD phytoplasma in Sri Lanka was determined for the first time. Sugarcane phytoplasmas of the 16SrXI group showed a clear separation from the 16SrXIV group.

In silico RFLP analysis indicated variations in restriction sites between 16SrXI and 16SrXIV phytoplasma groups, suggesting close association of WCLWD and the AYLD phytoplasmas with BGWLD phytoplasma of the 16SrXIV group, which was reiterated by F values ($F = 1.0$). Nonetheless F values among WCLWD, AYLD and SCWLD was

0.56, indicating dissimilarity among these sequences. The F between SCWLD and SCGSD was calculated to be 1.0.

Partial sequences of *secA* of WCLWD, AYLD, BGWLD and SCWLD phytoplasma populations revealed 6, 2, 3 and 0 polymorphic sites respectively. The highest Hd was found in WCLWD (0.8 ± 0.074) followed by BGWLD (0.727 ± 0.109) and AYLD (0.511 ± 0.164). Comparatively low nucleotide diversity was observed in all three phytoplasma populations. The π values were greatest in WCLWD ($0.00818 \pm 0.00113_{SD}$) > AYLD ($0.00254 \pm 0.00093_{SD}$) > BGWLD ($0.00436 \pm 0.00112_{SD}$) phytoplasmas. k values too showed this same trend; WCLWD isolates ($k = 2.152$) > while isolates of AYLD and BGWLD showed $k = 0.667$ and 1.152 , respectively.

Tajima's D test resulted in 0.58265, 0.18393 and -0.63919 for WCLWD BGWLD and AYLD strains respectively, that indicated a non-significant deviation from neutrality.

Low F_{ST} values between sub populations of WCLWD/ AYLD (0.08238) and WCLWD/ BGWLD e (0.03004), while significant genetic differentiation between any two sub populations was observed between SCWLD/SDGSD and the other phytoplasma populations ($F_{ST} = 0.9$) tested.

DISCUSSION

The disparity between 16S rDNA based and *secA* based phylogenetic analyses of WCLWD phytoplasma may have resulted due to several reasons. The superlative hypothesis would be that this is a new strain having genes common to both 16Sr XI (16S rDNA) and 16SrXIV groups (*secA*), suggestive of horizontal gene transfer between co-infecting strains, thus having evolving as a new species.

Importantly, this study for the first time established the existence of AYLD in Sri Lanka. Thus, identification of arecanut as an alternative host of WCLWD phytoplasma will increase the complexity of epidemiological studies.

This prototype study on population genetics of phytoplasma strains in Sri Lanka deduced that in relation to the *secA*, all the phytoplasma populations had very low genetic diversity that may have resulted due to low mutagenic incidences. The homogeneous nature of these populations is important in implementation of control measures, as all individuals of such populations tend to respond in the same manner.

ACKNOWLEDGEMENT

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A community 'Dieback Information Delivery Management System'—DIDMS

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INTRODUCTION

Project Dieback has been an ongoing ten year Western Australian state NRM Office and federal government funded project to strategically address the threat of *Phytophthora cinnamomi* (h *P. cinnamomi*) to the state's vulnerable biodiversity.

The occurrence, distribution and ongoing threat of *P. cinnamomi* does not discriminate between differing land tenure, and land managers/owners.

Effective management of *P. cinnamomi* requires an open and transparent exchange of information between stakeholders.

The online Dieback Information Management and Delivery Information System (h DIDMS) is a web-based GIS and database that facilitates the sharing of *P. cinnamomi* information.

MATERIALS AND METHODS

DIDMS has been constructed by Gaia Resources using their online 'Geographic Reporting Information Database.' (1) (GRID)

DIDMS requires people to register online as either an external or internal user. *Internal* users are registered Pc interpreters with the Department of Park and Wildlife (h DPaW). *External* users are all other people who are not registered Pc interpreters.

Once a user has registered with DIDMS they can access four components: *add and edit data*, *view data*, *mapping and reporting* (2) and a *user manual* (3).



Figure 1. Layout components of the DIDMS webpage.

DIDMS's data structure is divided into *activities*, *baselayers*, and *user layers*. Activities consist of standardised data templates for users to input disease status information, such as point source samples or area interpretation. Other data that can be added includes various management and planning activities. The activity data can be shared and viewed by users depending on their category as internal or external (2).

Baselayers is view-only data and includes point and area disease status, vegetation susceptibility, imagery, climate, hydrology, vegetation, roads, DPaW tenure and indicative Top 100 State Priority Protection Areas (3).

User layers is added personal data that can only be viewed by the user. This data may include GPS survey data, imagery and rare flora shapefiles (2).

Detailed login and data download disclaimers have been designed to protect users from misusing data in DIDMS. The disclaimers outline how to use *P. cinnamomi* disease data based on currency, scale and other quality parameters (3).

RESULTS

DIDMS enables users to create and modify data online using standardised templates, produce simple automated maps, and store data collectively. The templates can be downloaded for desktop GIS bulk data management.

There are more than 100 registered DIDMS users ranging from state and local government, regional NRM groups, community groups, private sector, universities, and schools. Online self-training is available within the DIDMS site with the user manual, but additionally up to 60 people have received formal DIDMS training.

The categories of internal and external users means that disease data can be quality assured and managed by setting permission levels as to what data a user category can view or download. It allows for a diverse array of expert and non-expert stakeholders to view, store and share information (2).

Some baselayers within DIDMS have been linked to a Dieback public map page for general viewing of basic data (2). This provides a quick and easy viewing platform of general statewide datasets linked to DIDMS.

DISCUSSION

DIDMS provides a safe repository to store and share *P. cinnamomi* information. The more data stored within DIDMS, the more useful it is for management planning.

Further refinement and additional functionality built into DIDMS will be required in the future as registered users become more familiar with the tool.

ACKNOWLEDGEMENTS

Piers Higgs, Gaia Resources.

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Utilising citizen science to monitor *Corymbia calophylla* (Marri) canker incidence and severity

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INTRODUCTION

Corymbia calophylla (marri), a keystone tree species of woodlands and forests in the southwest of Western Australia, is suffering a major decline syndrome associated with the canker fungal pathogen *Quambalaria coyrecup* and the foliar pathogen *Q. pitereka* (1).

Marri is an iconic tree species and plays a major role as a food source, habitat tree and refugia for numerous vertebrate and invertebrate fauna including the endangered Carnaby's cockatoo (*Calyptorhynchus latirostris*), as well as being a key species for southwest beekeeping and honey production.

Consequently, the impact of the pathogen on marri is causing increasing concern across the community, and there is substantial support from these communities to develop methods to manage these pathogens in the landscape.

We have developed a smartphone application that can be used by citizen scientists to capture GPS location, incidence and severity of cankers on trees and lodge photographs and other site information to a central data repository, providing valuable additions to our current knowledge on the incidence of this disease.

MATERIALS AND METHODS

The Marri App was developed collaboratively by the Eastern Metropolitan Regional Council, Murdoch University and Gaia Resources. The Marri App helps citizen scientists to identify and record disease symptoms on marri, with embedded fact sheets providing users with images and information on the identification of cankers and shoot blight (Figure 1). The app can also be used to record the presence of healthy trees, adding further information to the database.

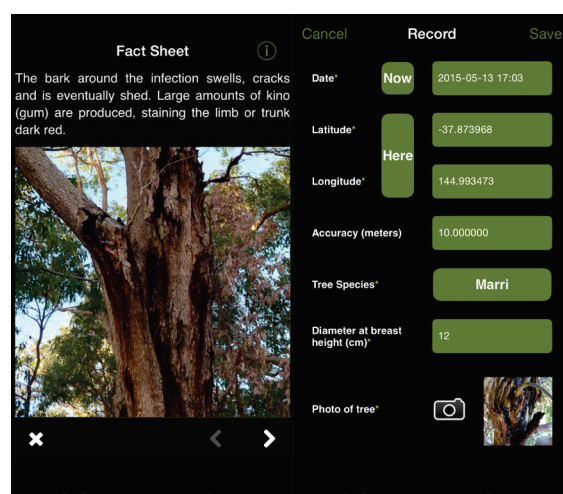


Figure 1. The Marri App: screenshots illustrating aspects of the fact sheet and data recording functionality.

RESULTS

Since its launch in December 2013, 138 registered users have contributed 366 observations from a range of regions across the southwest of WA (Figure 2). These comprise 335

trees across 31 sites. Over 300 images have also been submitted, providing documentation of the disease, a reference point for correct diagnosis, and a potential baseline for measuring the outcomes of subsequent treatments.

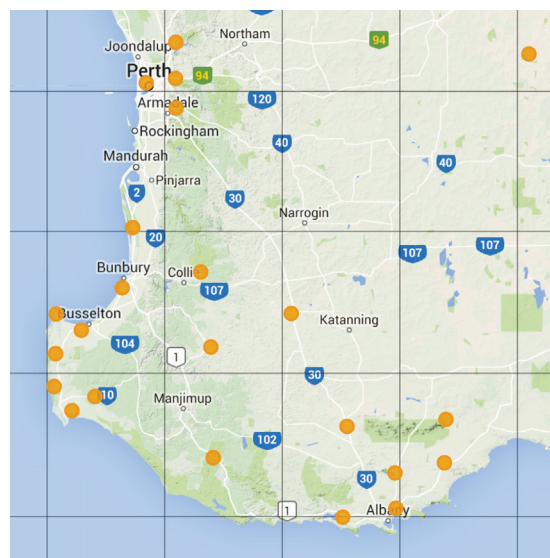


Figure 2. Locations where the Marri App has been used to collect information on marri canker presence.

DISCUSSION

The development of this innovative app has provided ongoing engagement with concerned members of the community in both urban and rural regions. More than just education and outreach, it has allowed a large scale data collection approach that would otherwise be a laborious and costly research problem.

The contributed data will be used by research scientists to understand the decline process and find treatments with the aim of developing sustainable management solutions.

ACKNOWLEDGEMENTS

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Learning and teaching academic standards statement for agriculture—how does plant pathology fit?

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INTRODUCTION

The Australasian Plant Pathology Society at various meetings over the years has discussed the urgent need to ensure improved capacity in agricultural industries, particularly through the training of plant pathologists. There are two main issues facing our discipline within the context of agriculture and agricultural education:

- The inability to attract students to work in agriculture and related industries, let alone as plant pathologists. In Australia, recent studies by the Australian Council of Deans of Agriculture have shown that there are 2000 jobs available annually in agriculture (including those jobs that service agriculture such as consultants and plant pathologists), but only between 600 and 800 graduates per year in agriculture and related disciplines. Anecdotal evidence (conversations with academics) suggests that New Zealand and other neighbours also find it difficult to maintain enrolments in this discipline.
- No consolidated definition of what skills we (industry, academics, students and practitioners) regard as absolutely essential before we let our students into the real world. We all acknowledge that we want students that can deal with the very complex problems facing us in food, fibre and fuel production in a world of climate change, but how they can be benchmarked nationally and internationally remains to be fully determined.

In addition to this, at least in Australia, we are faced with a new regulatory framework that will require all Universities to benchmark and show alignment of their curriculum with learning outcomes that is demonstrated through appropriate assessment. We led a project supported by the Office of Learning and Teaching that has created an agriculture-specific set of threshold learning outcomes (TLOs) and associated academic standards statement (The AgLTAS project). The AgLTAS statement is a formal document that articulates the collective views of agriculture discipline stakeholders, including academics, industry and students, endorsed by the Australian Council of Deans of Agriculture. We are now using the TLOs in a case study to benchmark curriculum at two Universities (the University of Tasmania and the University of Adelaide). The curriculum mapping process has allowed us to reflect upon the place of individual disciplines, such as plant pathology, in ensuring graduates of agriculture degrees have met the TLOs.

MATERIALS AND METHODS

In total, 21 workshops and two online surveys were undertaken nationally to inform the preparation of the AgLTAS statement by the project team and reference group. The consultative and reiterative process has been described elsewhere (1). Workshops and consultations were then held with academic staff at both institutions to map curricula against the TLOs for agriculture using an online curriculum mapping tool (CMT). A reflective process has then been used to collate information related to the major discipline

of plant health (plant pathology, nutrition and related topics).

RESULTS AND DISCUSSION

The five TLOs presented in the AgLTAS statement reference Knowledge; Understanding; Inquiry and Problem Solving; Communication; and Personal and Professional Responsibility. Discussions with industry stakeholders that need access to graduates specialising in plant pathology/plant health suggests that industry requires individuals that not only have a broad knowledge of pathogens and associated basic diagnostic skills, but that they also require a skill set that allows them to make decisions when faced with a real world problem that involves economics and environmental implications also. Some components of the TLOs are therefore strongly contextualised to the highly multidisciplinary nature of agriculture, capturing diverse elements such as business and social contexts, adoption and the value chain (2). Industry also agreed that students needed to demonstrate highly developed problem solving and communication skills. However, industry-specific (vocational) knowledge was generally regarded as attainable during on-the-job training after graduation.

Agriculture and related disciplines are offered in 14 Australian universities (as a three- or four year specialist degree or as a major in a science degree). Curriculum mapping at the two Universities in this project revealed strong interactions with industry and curriculum that meets or exceeds expectations. Both Universities are using the reference to the TLOs as an opportunity to refresh curriculum and refine certain sub-disciplines, including plant pathology. We hope that other academics would find the information to be presented useful in benchmarking their teaching of agriculture and related sub-disciplines such as plant pathology.

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Holy moley Grainman (Batman)! What's that? Can our farmers and agronomists recognise pests and diseases in grain crops?

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INTRODUCTION

In 2000, Plant Health Australia was established to be the national coordinator of the government industry partnership for plant biosecurity in Australia. This means that they work to promote biosecurity practices to minimise the impact of high priority pests and diseases (HPP's) on Australia's agricultural and horticultural industries and the general community and to enhance market access. This presentation will focus on the grains industry, and the grains farm biosecurity program. This program was established to raise awareness and improve biosecurity practises on-farm. As part of this program, Grains Biosecurity Officers have been funded by industry and were established in five States within Australia to help farmers improve their on-farm biosecurity and maintain awareness of the HPP's within the grain industry.

So how do we know if this program is being effective? Can farmers and agronomists in Western Australia and Victoria recognise the top four HPP's that could occur in the grains industry? The aim of this research is to determine if farmers and agronomists can recognise the HPP's and make recommendations as necessary.

MATERIALS AND METHODS

A questionnaire was developed to determine if growers and agronomists could identify the top four HPP's within the grains industry. Four questions were used, listing associated and non-associated symptoms with each of the individual HPP's. Participants were asked to choose the associated symptom, and were also able to indicate if they did not know for that HPP. The four HPP's used in the questionnaire were Karnal Bunt, Khapra Beetle, Barley Stripe Rust and Russian Wheat Aphid. Participants were also asked if they had sent samples into the National Exotic Plant Pest Hotline.

The questions were part of a larger survey that was sent out in March 2014 to farmers and agronomists. The survey was distributed in two formats—as an online survey in Qualtrics (Qualtrics, Provo, UT) and as a paper based survey handed out at six regional Crop Update meetings within WA and posted to members of the Birchip Cropping Group in Victoria.

The data was analysed to determine if there were any significant differences between growers and agronomists in the identification of the HPP's by crosstabs and Pearson's Chi-Square test. Further analysis was conducted to determine the influence of age, gender, education level and State. All statistical tests were done in SPSS (Statistical Package for the Social Sciences (SPSS) IBM ver. 22. Surveys with incomplete demographic data were not included in the results.

RESULTS AND DISCUSSION

A total of 248 useable surveys were returned with 135 grain growers and 113 agronomists within Australia.

For each of the HPP's the number of agronomists who identified all of the symptoms correctly was significantly higher ($p < 0.01$) than the number of growers except with Barley Stripe Rust, where the numbers were similar. These results are similar to those collected by Hammond in 2008, who found there was a significant difference ($p < 0.05$) between growers and agronomists, and DAFWA staff in correctly identifying the symptoms associated with each of the HPP's (1).

In the current study a significant difference ($p < 0.05$) was found between the number of participants who correctly identified all of the symptoms for each HPP between Western Australia and Eastern Australia. This result suggests that the Grains Biosecurity Officers maybe using different communication methods to their Industry and will require further investigation.

Participants with a higher education level were more likely ($p < 0.05$) to be able to identify the two insect HPP's, Khapra Beetle and Russian Wheat Aphid. A high proportion of all participants were not able to correctly identify any of the characteristic symptoms associated with the HPP's.

There was no significant difference ($p < 0.05$) between age groups and the ability to identify the four different HPP's.

In conclusion, the results show that when designing information to raise awareness in the grains industry about HPP's, demographic information needs to be taken into account and a variety of methods need to be used to communicate the biosecurity message.

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Experience of a lifetime—plant pathology and IDM in Laos

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ABSTRACT

As a volunteer plant pathologist with the Champasak Provincial Crop Disease Diagnostics Laboratory in Pakse, southern Laos, no two days were ever the same—surveying in cabbage fields in the pouring rain, laboratory diagnostics, teaching plant disease theory in Lao-glish and dining on fried fish and frogs was all in a week's (and sometimes day's) work. I worked with incredible people in an incredible location with incredible plant diseases! And what a training ground! I surveyed more than 20 different crops during my 18 months in Laos, saw more than 50 plant diseases, diagnosed at least 15 of these and worked with at least three biosecurity threats to Australian plant industries and ecosystems!

But, there is a serious development aspect to this story. Vegetable production is a key agricultural industry both for smallholders and business owners in Laos, with a growing and profitable regional export market. Despite this, there has been virtually no extension support for plant disease management. Over the past six years of intensive production there has been a gradual increase in the incidence and severity of diseases, leading to a decline in productivity. The decline in head cabbage yields (the main export vegetable crop to Thailand), provides a good example of this problem. The cabbages have been grown in monoculture, two to three crops per year, using transplants grown in plots in the same fields over these six years. Under these conditions, yields have declined dramatically in older fields, with up to 50% losses. The main cause of yield loss in head cabbages has been collar rot, caused by *Rhizoctonia solani*. Other major vegetable disease problems in southern Laos include: Sclerotinia head and stem rot (*Sclerotinia sclerotiorum*) on cabbage, Bacterial wilt (*Ralstonia solanacearum*) on tomato, Late blight (*Phytophthora infestans*) of tomato and Root Knot Nematode on a wide range of crops.

A major workshop, 'IDM of Vegetables in Lao PDR—Train the Trainer', supported by the APS Office of International Programs, was conducted in September 2014 to address the lack of understanding of disease diagnostics, integrated disease management (IDM) and basic crop agronomy by both district staff and small-holder farmers, through a 'train-the-trainer' educational format. Lao-language training materials were developed and delivered in a workshop involving crop/field visits to train district staff and key small-holder farmers. The long-term aim is to facilitate the extension process by local staff, as well as farmer to farmer information transfer to reduce head cabbage losses caused by pathogens such as *R. solani*, through integrated disease management.

Together with Scope Global, the Crawford Fund of Australia, mentor Lester Burgess, current volunteer Sophia Callaghan and a number of other Plant Health volunteers, we are working to provide a bright future for Laos agriculture through capacity building in crop disease diagnostics, integrated disease management (IDM), pest diagnosis and integrated pest management (IPM). Crawford Fund is

pivotal in ensuring our work is successful—supporting workshops in Integrated Disease Management, Plant Disease Diagnostics, Bacterial Diagnostics and IDM, and Integrated Pest Management.

Strong relationships have been built with the International Collection of Microorganisms of Plants (ICMP) in New Zealand, to allow for accessioning of isolates of key pathogens, and with other plant pathology experts—such as Italian Bakanae experts Drs Virgilio Balmas and Alessandro Infantino (see their poster this conference!), Asian vegetable pathology expert Dr Len Tesoriero, nematologist Dr Jenny Cobon, bacteriologist Dr Ric Cother and citrus pathologist Nerida Donovan, to name a few. Australian plant pathologists are well placed to provide good quality and relevant support to plant pathology and IDM efforts in Laos—we grow many similar crops under similar conditions. I encourage all interested parties to get in touch with Lester Burgess to keep abreast of future opportunities to assist capacity building in plant pathology and IDM in Laos—I guarantee it will be a life-changing and worthwhile experience!

ACKNOWLEDGEMENTS

The authors would like to acknowledge the incredible support of Scope Global (ICM David Sharman-Selvidge in particular) and the Australian Government's Australian Volunteers for International Development (AVID) Program. Financial, media and mentoring support from the Crawford Fund of Australia is gratefully acknowledged. Financial and library building support from the American Phytopathological Society Office of International Programs is likewise gratefully acknowledged. Many thanks to the many volunteers, on ground and as part of the online mentoring team, who have assisted our efforts—including Dr Bevan Weir and Maureen Fletcher from ICMP and the plant pathologists listed above. And a special and heartfelt thanks to local PAFO staff and friends in Lao PDR—in particular to Mr Gerry Duckitt, translator extraordinaire.

The plant health RD&E investment plan for the Australian vegetable industry

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INTRODUCTION

Plant health and crop protection projects constituted over 50% of research, development and extension investment by the vegetable industry from 2001-2010. It is recognised within the current Vegetable Industry Strategic Investment Plan that continued investment in this area is important.

Whilst continued investment at previous levels is not likely due to changing industry priorities there remains an ongoing requirement for plant health and crop protection research, development and extension in the areas of greatest need and of highest potential benefit, especially innovative technologies and integrated systems. The vegetable industry required a well-founded plan for RD&E investment in this area to ensure that resources were invested cost effectively.

RMCG have developed a prioritised Plant Health and Crop Protection Research, Development and Extension (RD&E) Plan for the Australian levy vegetable industry under Horticulture Australia project VG12048—Plant Health Desktop Study.

MATERIALS AND METHODS

The RD&E Plan was developed based on an agreed framework and following a review of previous levy funded RD&E outputs, a situation analysis (available information and data, relevant aspects of production systems, extension and adoption), discussions with growers, advisers, researchers and agribusiness, and a review of new technologies. Soil condition management RD&E was included at all stages.

RESULTS

In spite of extensive research in the area of plant health, some R&D gaps and barriers to research and development (R&D) adoption were identified. Many growers do not have the time to review large R&D project reports, projects may not necessarily develop practical applications or resources for growers, or project outputs may not be presented to growers in a form that allows easy adoption or adaptation. Information needs and capacity to adopt new practices varies amongst growers due to many factors, which were identified by the project; these factors and their implications for adoption are often neglected in R&D extension.

The Plant Health Desktop Study considers economic principles, the current industry outlook, and addresses barriers to research adoption. It recommends a focus on exploring new technologies and techniques as well as effective extension of previous industry wide programs. Plant health and crop protection RD&E programs for levy vegetables should be oriented towards industry needs across the supply chain, with a multidisciplinary approach that considers major production systems and how they may evolve in key vegetable growing areas. Research, development and extension capabilities need to be used collaboratively.

Programs and activities should build on previous work and be less ad hoc, fragmented or repetitive. Grower participation and demonstration of R&D outcomes should

be an important part of applied research. Efficiency and effectiveness of R&D and especially extension can be improved through linkages between sectors and organisations and the delivery of regional and local programs. Synergies between goals and activities of government programs, departments, universities, NRM bodies, agribusiness and independent advisers should be explored and used for the benefit of growers and the entire supply chain.

The RD&E investment plan. The RD&E Plan provides clear direction, strategies and recommendations towards an integration of RD&E while at the same time considering special needs for future RD&E. This includes regard for issues such as acute or chronic plant health problems, and regional or specific grower cluster needs. The main directions are.

The vegetable industry requires a coordinated and cohesive approach that focuses on **integration on many levels** including:

- Development and dissemination of integrated plant health knowledge based on requirements for the management of vegetable production systems rather than driven by discipline (e.g. soils, pests, diseases, water, nutrition)—**taking the Plant and Crop View**
- Inclusion of aspects related to production economics, market demands and business decision making—**taking the Grower View**
- **Inclusion of specialists from relevant technical disciplines** in the undertaking of R&D (e.g. pathology, soil science, crop physiology, nutrition)
- Utilisation of specialist **skills associated with the delivery and evaluation of RD&E** (especially extension and evaluation).

Four key RD&E investment areas and levels (% of available RD&E funds) for vegetable plant health and crop protection, as well as subprograms for each of program area, were identified in the Plan for the subsequent 5 years. The Plan also provides guidance on development and prioritisation of programs or projects, specific activities, and the measurement and evaluation (M&E) of outputs and impacts, as well as effective reporting to all stakeholders.

ACKNOWLEDGEMENTS

The project was funded from vegetable industry levies with matched contributions from the Australian Government via Horticulture Australia Limited. Ben Callaghan was the responsible project manager.

Julian Morison from EconSearch Pty Ltd produced the economic impact analysis model.

Paul Horne and Jessica Page from IPM Technologies contributed to the project framework and the formulation of extension principles.

REFERENCES

The final report for project VG12048, Plant Health Desktop Study, available from Horticulture Innovation Australia Limited, lists all references used for the review and preparation of the Plant Health and Crop Protection RD&E Investment Plan.

Impact of the foliar pathogen *Phytophthora pluvialis* and soil borne pathogen *P. cinnamomi* on *Pinus radiata* root development in controlled conditions

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INTRODUCTION

At least 3 foliar and 13 soil borne *Phytophthora* pathogens are associated with *Pinus radiata* globally. Little is known of how each of these species differentially impact root development. It can also be difficult to measure roots and control the rhizosphere environment. This research aimed to develop a bioassay to quantify root and disease development in *P. radiata* over time; and to compare inoculation with *P. pluvialis* zoospore suspensions, *P. pluvialis* colonised miracloth discs (CMD) and *P. cinnamomi* CMD, to infect and cause root disease.

MATERIALS AND METHODS

Clonal growth in seedling pouches. Seven *P. radiata* clones were grown in seedling pouches (CYG-Mega International), comprised of an inert transparent pouch and wicking media (1). Foliage and root health was assessed fortnightly for eight weeks, using the WinRhizo root analysis system (2).

Root health and infection in seedling pouches. After the initial assessment of root production; the root systems of the seven established *P. radiata* clones were inoculated with *P. pluvialis* zoospore suspensions, *P. pluvialis* colonised miracloth discs (CMD) and *P. cinnamomi* CMD. The impact of inoculation on root health was measured fortnightly for six weeks, using WinRhizo. The isolates of each species used, were recovered from *P. radiata*. The roots of all seedlings were harvested six weeks after inoculation and plated onto PRHN media to determine the extent of *Phytophthora* infection. Data was analysed using Statistica version 12 (Dell 2015).

RESULTS

Each *P. radiata* clone differed substantially in its establishment of roots within the seedling pouch system (Figure 1). *Phytophthora pluvialis* inoculations caused substantial loss of fine roots between 0 to 0.5 mm in diameter compared to control treatments, six weeks after inoculation (Figure 2); however, no treatment effect could be detected for roots greater than 0.5 mm in diameter. *Phytophthora* isolates were recovered from most treated plants. Inoculation with *P. pluvialis* zoospores or CMD or *P. cinnamomi* did not result in significant variation in root loss between 0 to 0.5 mm in diameter.

DISCUSSION

Our findings demonstrate that under experimental conditions, the seedling pouch bioassay is an effective system to accurately measure root development over time and that different *P. radiata* clones produce roots at different rates. Under these conditions *P. pluvialis* is capable of infecting *P. radiata* and causing fine root loss between 0 to 0.5 mm in diameter. Results also show that zoospores and CMD were both effective inoculation methods with comparable inoculations between methods and pathogen species.

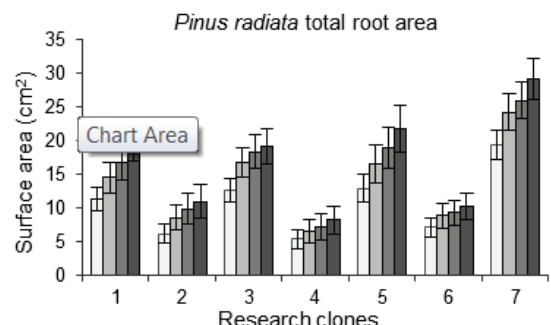


Figure 1. Mean root area ($\pm$ standard error) ($n = 60$) of seven *Pinus radiata* clones; () two, () four, () six and () eight weeks after being transferred into seedling pouches, from root instigation media.

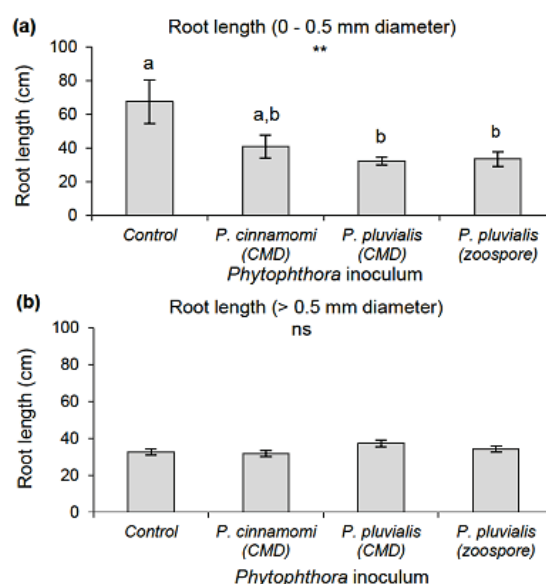


Figure 2. Mean root length ($\pm$ standard error) of *Pinus radiata* seedlings grown in seedling pouches, six weeks after inoculation with: *Phytophthora cinnamomi* Colonised Miracloth Discs (CMD); *P. pluvialis* CMD; *P. pluvialis* zoospore suspension; or non-inoculated control treatments. Root length was measured for root diameters 0–0.5 mm (a) and diameters > 0.5 mm (b). ** = $P \leq 0.01$, ns = not significant. Small letters denote the results of the *post hoc* test (Bonferroni) and bars with the same letters are not significantly ($P \leq 0.05$) different.

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Age-related susceptibility of *Eucalyptus* species to *Phytophthora boodjera* prov. nom

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INTRODUCTION

Since 2011, damping-off and seedling mortality were observed in nurseries producing mallee eucalypts in large quantities for revegetation projects within the wheat-belt of Western Australian (WA). The causal agent of this disease was identified as *Phytophthora boodjera* prov. nom. Symptoms included stunting of growth, which was often not observed until seedlings reached the 4-6 true leaf stage. Losses because of this new disease were substantial and a short fall in continuous supply of these seedlings was a concern for the industry. Additionally, the distribution and the origin of *P. boodjera* remains unknown, thus requiring the precautionary principle to be invoked by land management groups not wishing to introduce an unknown pathogen into their environmental plantings. This in turn put sudden additional pressure on nursery owners to produce *Phytophthora*-free seedlings when the source of contamination of the nursery was unknown. The main issue was *P. boodjera* only a damping-off pathogen or can it infect and damage older seedlings and/or trees? This study addresses the question by evaluating the age-related susceptibility of five species of *Eucalyptus* to *P. boodjera*.

MATERIALS AND METHODS

This study evaluated the age-related susceptibility of five species of *Eucalyptus* (*E. polybractea*, *E. kochii* subsp. *plenissima*, *E. kochii* subsp. *borealis*, *E. loxophleba* subsp. *lissophloia*, *E. loxophleba* subsp. *gratia*) to six isolates of *P. boodjera* and three isolates of *P. arenaria* in sterilised washed river sand-infestation pot trials. *P. cinnamomi* was included for comparison. *Eucalyptus* spp. were inoculated with all *Phytophthora* isolates at 0, 2, 4, 12 and 88 weeks post-germination. The following measurements were included in data sets where applicable: number of seedlings germinated, height of seedlings, root length and dry root weight.

RESULTS

Pre-emergent mortality was almost 100%. Post-emergent mortality was 50-100% depending on isolate. Mortality was also high for 1-month old seedlings (46 to 68%) and root length of surviving seedlings was severely reduced. Death from root infection was not observed for seedlings inoculated at 12 and 88 weeks, but this resulted in root necrosis and reduced root dry weight compared to non-inoculated controls.

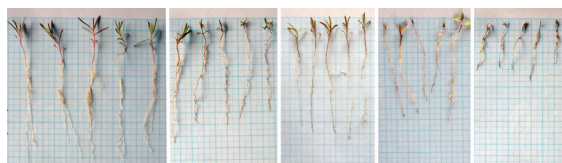


Figure 1. Comparisons of root length and damage of *E. kochii* ssp. *plenissima* 5 weeks after inoculation; (left to right) non-inoculated control, *P. cinnamomi* MP 98-48, *P. arenaria* ENA1, *P. boodjera* VHS 26806 and VHS 237382. Seedlings were inoculated 4 weeks after sowing.



Figure 2. Comparisons of root length and damage of *E. loxophleba* ssp. *gratia* 12 weeks after inoculation; (left to right) non-inoculated control, *P. cinnamomi* MP 98-48, *P. arenaria* ENA1, *P. boodjera* VHS 26806 and VHS 237382. Seedlings were inoculated 12 weeks after sowing.



Figure 3. Root reduction of *E. kochii* ssp. *plenissima* inoculated with different *Phytophthora* and isolates at 88 weeks. Whole root systems were visually rated for root rot on a scale 1 to 4 (1= no damage, 4= severe root damage).

DISCUSSION

Phytophthora boodjera is a pre- and post-emergent pathogen of mallee eucalypts. These eucalypts are susceptible to *P. boodjera* at all life stages tested, but the mortality rates declined with seedling age.

Endemic *Phytophthora* species in their natural ecosystems will have coevolved with their hosts, and are not expected to cause severe disease. When conditions change, however, although formerly benign, the pathogen will later develop as aggressive and destructive (1). The emergence of new species of pathogens can result in new disease epidemics that can be ecologically and economically difficult to manage, and also has serious implication for biosecurity (2,3). *P. boodjera* is a pathogen to young mallee eucalypts with the likelihood of death decreasing as seedlings age (except if there is a flooding event). The events leading to its recent appearance in the nurseries remain unknown and further investigations are underway to determine if this is an introduced or endemic pathogen.

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Foliar inoculation of *Agathis australis* infected with the soil borne pathogen *Phytophthora agathidicida*

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INTRODUCTION

Agathis australis (D.Don) Lindl. ex Loudon (1829) (kauri) is one of NZ's most economically and culturally important native tree species. These majestic trees are part of NZ's rich history and are valued by Māori as a connection to their spiritual beliefs and ancestors. Kauri is under serious threat from the soil borne pathogen *Phytophthora agathidicida*, which causes kauri dieback disease. Increasing levels of kauri dieback disease have been reported in the upper North Island (1). In order to protect and restore kauri in *P. agathidicida*-infested areas, natural resistance needs to be identified, for future planting. The overall aim of this research is to develop a fast and efficient assay that will detect differences in resistance to *P. agathidicida* in different kauri propagation lines. The aims of the work described here were to (a) develop a pathogenicity assay system using detached kauri leaves for ex situ screening of susceptibility, and (b) determine if any variation in the susceptibility of kauri clones to *P. agathidicida* can be identified using leaf inoculation.

MATERIALS AND METHODS

Six, five-year-old kauri clones from Holts Forest (north island New Zealand) were selected for this experiment. Forty fully-formed leaves from the current year's cohort were excised from each clone and inoculated with three different *P. agathidicida* isolates (P1, P2, P3). Isolates were obtained from different locations within the kauri forests in New Zealand, and used for controlled inoculations, using a modified version of the method of Horner and Hough (2). Six days post-inoculation, foliage health was assessed using the WinFolia analysis system (Regent Instruments Inc. Canada). The leaves were plated onto 10% carrot agar base with PRNH antibiotics, modified from Hüberli (3). Data was analysed using Statistica version 12 (Dell Inc. 2015, Texas, USA).

RESULTS

Variation in lesion area was observed among the six clones infected with the three *P. agathidicida* isolates (Figure 1). Clone C4 had the largest average lesion area of 57%, followed by clone C5 which exhibited the second largest lesion area of 23%. The control inoculation also resulted in some lesions. Inoculations with the three *P. agathidicida* isolates also resulted in substantially different lesion development. Isolate P1 was the most virulent, while P3 was the least. *Phytophthora agathidicida* was successfully reisolated from most inoculated leaves, excluding the control treatments (Figure 2).

DISCUSSION

These findings demonstrate differences in resistance among kauri clones when infected with different isolates of *P. agathidicida*. The findings also reinforce that *P. agathidicida* is an aggressive pathogen of kauri that has the ability to infect leaves under artificial conditions. Although the control treatments produced visible lesions, this is most likely a wound response that was made during inoculation. In addition, *P. agathidicida* was not recovered

from any of the control treatments. Some isolates were more virulent than others. Additional root and leaf assays are being conducted on different clones in order to develop a meaningful assay that is representative of natural infection in kauri forests. Further research is needed to determine the level of resistance in other kauri genotypes when infected with *P. agathidicida* under natural conditions.

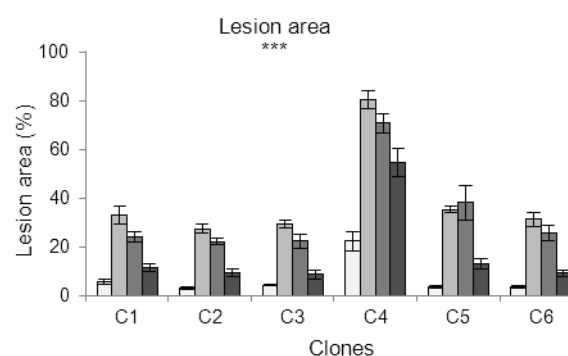


Figure 1. Average percentage lesion area ($\pm$ SE) on foliage for six *Agathis australis* clones infected with three *Phytophthora agathidicida* isolates control (), P1 (), P2 (), P3 (), six days after inoculation. Statistics are for univariate tests where the multivariate main effect is significant. *** $P \leq 0.001$.

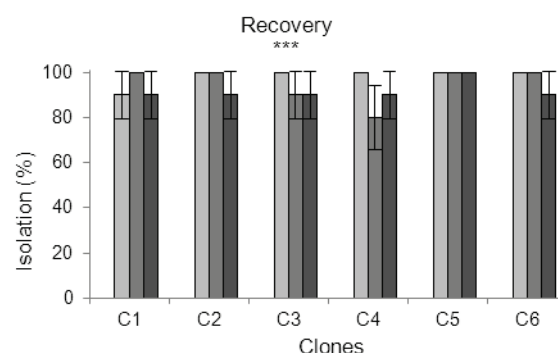


Figure 2. Average percentage recovery ($\pm$ SE) for six *Agathis australis* clones infected with three *Phytophthora agathidicida* isolates control (), P1 (), P2 (), P3 (), six days after inoculation. Statistics are for univariate tests where the multivariate main effect is significant. *** $P \leq 0.001$.

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Variation between isolates of *Neofusicoccum* species in pathogenicity and sporulation on grapevine shoots

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INTRODUCTION

Botryosphaeriaceae species are important pathogens worldwide, causing cankers and dieback on a wide range of agricultural, horticultural and forestry plant hosts, including grapevines. However, incidence reports of the most common species on grapevines differ between countries. Further, the pathogenicity studies in different countries have reported differences in lesion lengths and sporulation (1-4). In New Zealand vineyards, sampling from symptomatic wood tissues showed that the most common and pathogenic *Botryosphaeriaceae* species were *Neofusicoccum luteum* and *N. parvum* (1, 4). In this study, multiple vineyard isolates of these two species were tested for pathogenicity and sporulation so that reliable isolates might be selected for future research.

MATERIALS AND METHODS

Soft and semi-hard green shoots (~30 cm) of Sauvignon Blanc were harvested in late summer and their bases held in water until they were inoculated (1-3 d). The single spore isolates used had been stored at -80°C and were revived by subculture on potato dextrose agar. The base of each shoot was inserted into a water container and it was centrally inoculated (1) with one of the isolates of *N. luteum* (six isolates) or *N. parvum* (eight isolates). The six replicates per isolate and shoot type were randomly arranged in a laboratory under natural light with frequent misting for the first 4 days. For each treatment, two replicates (blocks) were set up per day on three consecutive days. The lesion lengths were measured using a digital calliper after 7 d for soft shoots and after 14 d for the hard shoots.

The stem pieces containing the lesions were removed, surface sterilised with 70% ethanol and air dried. A central, 1 cm section was cut from each lesion and placed in a separate bottle with a moist filter paper strip to provide high relative humidity, and held at room temperature (20-24°C) for 24 h, which allowed conidia to ooze from the pycnidia. Sterile tap water (2 mL) was added to each bottle and it was shaken vigorously, to suspend the conidia. Conidia were counted with a haemocytometer slide (three replicate counts). Data were analysed by ANOVA.

RESULTS

The lengths of lesions showed a block effect ($P=0.001$), with those inoculated on the day of cutting producing longer lesions than those held in water for 1-3 days before inoculation. However, the isolates followed the same relative trends on all days (blocks). For *N. parvum* mean lesion lengths for semi hard shoots were 3.52, 1.86, and 1.39 cm and for soft green shoots were 2.55, 1.51, and 1.30 cm, for day 1, 2 and 3, respectively. For *N. luteum* mean lesion lengths for semi hard shoots were 4.90, 1.70 and 1.45 cm and for soft green shoots were 3.42, 1.48 and 1.40 cm, for day 1, 2 and 3, respectively.

The isolate effect on lesion length was significant for soft shoots ($P=0.001$) but not semi hard shoots ($P=0.064$), and was significant for sporulation (conidia/mL) for both shoot types ($P=0.001$). Maximum differences between the most and least pathogenic isolates were 2.4 and 2.9 fold for

lesion lengths of *N. parvum* and *N. luteum*, respectively. For numbers of conidia released, maximum differences were 16.6 and 8.1 fold, respectively (Table1).

Table 1. Mean lesion lengths and numbers of oozed conidia from detached soft and semi-hard green shoots, showing isolates with the least and greatest values.

Isolates	Lesion length (cm)		Conidia x10 ³	
	Semi-hard	Soft green	Semi-hard	Soft green
<i>N. parvum</i>				
G69a1	3.81	2.93	104.4	55.6
MM562	2.80	2.65	67.6	48.2
B2141	1.63	1.28	8.2	7.3
A842	1.61	1.16	6.4	5.4
<i>N. luteum</i>				
CC445	4.25	3.90	86.2	57.6
ICMP 16678	3.56	2.52	80.9	55.8
A526	1.61	1.18	10.8	9.1
G31d2	1.48	1.14	9.0	7.1

DISCUSSION

In this study, fresher shoot held in water for 1 day developed longer lesions than those held in water for 3 days. This was unexpected as shorter lesions were reported (1) to develop in attached, actively growing shoots than in detached shoots. The isolates were consistently of greater or lesser pathogenicity with both shoot types, although soft shoot lesions developed more quickly than those on hard shoots. The isolate differences in sporulation observed in this study go some way to explain difficulties in production of conidia by these species which have been anecdotally reported by researchers (unpublished data).

ACKNOWLEDGEMENTS

We thank the Callaghan Fund for scholarship funding and Pernod-Ricard Ltd for providing practical support.

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Agrobacterium-mediated transformation of *Ganoderma boninense* to discern early stage pathogenesis of basal stem rot

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INTRODUCTION

The basidiomycete fungus *Ganoderma boninense* causes basal stem rot (BSR) in oil palm trees in tropical regions, and is considered to be the most economically devastating pathogen amongst major palm oil producers such as Indonesia and Malaysia. The disease initiated by infected wood debris establishes via root contact on host tissues following successful lignin degradation (1). Over the years, management practices have been widely employed with relatively poor efficiency whilst neglecting information on molecular basis for BSR pathogenesis. The disease is symptomless during the early stage of pathogenesis and renders diagnostic difficulty during disease screening of plantations. With the ever raising knowledge on gene function and expression, a suitable fungal transformation system would be able to resolve infection pathway and subsequent colonisation pattern of BSR in relation to host defense mechanism. Thus, the objective of this study was to develop a highly efficient protocol for tagged *G. boninense* that could ultimately resolve early stage pathogenesis of BSR.

MATERIALS AND METHODS

The *Agrobacterium* bacterial strains (LBA 4404, GV3103, EHA 101 and EHA105) were grown on Luria-Bertani (LB) medium amended with kanamycin as required. The mycelia was grown on MEA while the protoplast were regenerated on PDA supplemented with phosphate buffer pH 5.6 and KCl 0.6 M. Spore were harvested from matured fruiting bodies and stored as suspensions in deionised water. Minimal medium (MM) composed of 10 mM K₂HPO₄, 10mM NaH₂PO₄, 2.5 mM KCl, 2 mM MgSO₄·7H₂O, 0.7 mM CaCl₂, 9 µM FeSO₄·7H₂O, 4 mM (NH₄)₂SO₄, 10 mM glucose, pH 7.0 while induction medium (IM) was prepared as following; MM containing 0.5% (w/v) glycerol, acetosyringone, 40 mM 2-(N-morpholino) ethanesulfonic acid (MES), pH 5.3). Induction agar was prepared as induction medium with 2% agar and selection plate as glucose agar (variable concentration of glucose with 2% agar) supplemented with 100 µg/ml cefotaxime and 200 µg/ml Hygromycin B. *Agrobacterium* strains upon *vir* gene induction were co-cultivated with *G. boninense* on a nitrocellulose membrane for a period of 5 days at a temperature ranging from 20-28 °C on the induction plate. Upon co-cultivation, the nitrocellulose membrane was transferred to selection plate and number of individual colonies was recorded as transformants. PCR was performed to verify integration of *gus-gfp* fusion gene while real-time PCR was performed to determine gene copy number. Individual transformant colonies were directly used to infect rubberwood block which serve as inoculums for artificial infection of oil palm seedlings (2). At regular intervals (day 3, 6, and 12 after inoculation), root samples were harvested and assayed for *gfp* expression using confocal microscopy.

RESULTS AND DISCUSSION

A highly efficient and reproducible *Agrobacterium*-mediated transformation protocol was developed for *G. boninense*. The method was proved amenable to

protoplast, spore and mycelium of *G. boninense*.

Optimisation of co-cultivation conditions, transformant growth media, *vir* gene induction and *Agrobacterium* strains showed variable transformation efficiency ranging from 20-83%. The transformation efficiency was highest under a treatment combination of spore and LBA 1101 *Agrobacterium* strain with successful expression of hygromycin resistant marker gene and *gus-gfp* fusion gene, under the control of heterologous p416 *gpd* promoter. Other optimal transformation conditions included were *vir* gene induction in presence of 250 µM of acetosyringone, followed by co-cultivation at 22°C for 2 days and regeneration of transformants on glucose agar supplemented with glucose at 0.2 M/L and 0.6 M phosphate buffer. The expression of *gus-gfp* fusion protein was confirmed by PCR and gene copy number was found highly conserved. The expression of *gfp* was visible under the confocal microscope. The transformant maintained stable during mitotic division for three consecutive generations in absence of selection pressure and showed no morphological differences when compared to untransformed control *G. boninense*. Artificial infection of oil palm seedlings with transformant inoculums revealed initiation of disease at quaternary roots and tertiary roots. No detection of *gfp* was found on primary roots throughout day 0 – day 6 after inoculation. In contrast, though no visible symptoms were observed at quaternary roots at day 3 after infection, *gfp* fluorescence was found on the root surface. Latter, the *gfp* fluorescence was found in the cortex tissue and root vascular bundle on day 6 and 12 after inoculation respectively. The *G. boninense* is well characterised as lignin degrading fungi. However, BSR is established through non-lignified root structures followed by further progression into nutrient rich cortex tissue.

CONCLUSION

These findings which confer an efficient and reproducible *Agrobacterium*-mediated fungal transformation system would ultimately facilitate numerous interaction studies which are highly valuable for biotechnological management of BSR. Variability in oil palm root structure eases pathogenesis of BSR as demonstrated by selective infection site at non-lignified regions of the root tissues in oil palm seedlings.

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Does *Phytophthora* predispose Marri (*Corymbia calophylla*) to *Quambalaria coyrecup* canker?

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INTRODUCTION

Corymbia calophylla (marri) is a keystone species in the Jarrah (*Eucalyptus marginata*) forest in the south-west of Western Australia. Marri has been suffering a decline in health and vigour since the 1970s (1). Around the same time marri canker was reported and currently this canker disease is causing widespread mortalities. An endemic pathogen *Quambalaria coyrecup* is the cause of this canker disease (2), and the question was raised why would a supposedly endemic pathogen have such a devastating effect on marri? *Phytophthora* root diseases are cited as major causes of tree declines and death around the world, as they reduce the amount of water and nutrients that a plant can uptake. As both *Phytophthora* dieback risk (3), and canker incidence (2) are higher in anthropologically disturbed sites, this study aimed to investigate whether *Phytophthora* root infection might be associated with the incidence of canker disease in marri, thus increasing marri’s susceptibility to canker disease.

MATERIALS AND METHODS

Field surveys were conducted across the marri range. Five paired sites were selected, consisting of a natural forest site with an adjacent anthropologically disturbed site, such as a paddock or remnant land. Ten healthy and ten unhealthy (cankered) trees were selected on each of these ten sites. Soil samples were collected from the rhizosphere of these trees and baited according to CPSM best practice for the presence of *Phytophthora* species. In order to determine the distribution of the recovered *Phytophthora* spp across the different field sites, the Canonical Correspondence Analyses (CCA) method was used (Figure 1).

RESULTS

Four different *Phytophthora* species were isolated, *P. cinnamomi*, *P. multivora*, *P. aff ohioensis* and *P. elongata*. The numbers of *Phytophthora* species recovered and trees infected with *Phytophthora* were higher on the disturbed sites (Table 1). There was a higher incidence of *P. multivora* recoveries on disturbed sites (Figure 1).

Table 1. Recovery of *Phytophthora* isolates from healthy and cankered trees. For each combination of site status x tree health, the number of recovered *Phytophthora* spp. and the number of trees infected is indicated.

Site status	Tree health	N <i>Phyt.</i> spp.	N infected trees
Undisturbed	healthy	2	4
	unhealthy	1	2
Disturbed	healthy	4	11
	unhealthy	3	8

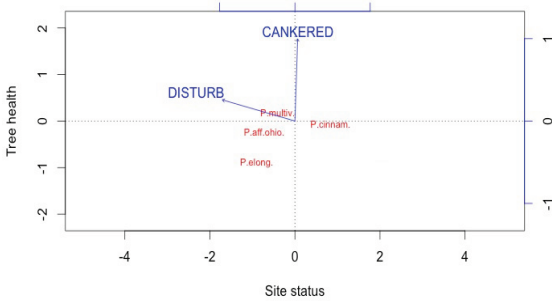


Figure 1. The distribution of recovered *Phytophthora* species. There was a significantly higher incidence of *P. multivora* recoveries on the disturbed sites.

DISCUSSION

These results indicate that, although *Phytophthora* spp. are associated with marri trees on all sites, the incidence of infected trees and recovered *Phytophthora* spp. were higher on disturbed sites. These results suggest that *Phytophthora* root infection can be associated with predisposing marri to the canker disease. Pathogenicity trials are being conducted to provide clearer evidence, quantifying the effect of *Phytophthora* root disease on the health of marri. A dual inoculation trial with *Phytophthora* and the canker pathogen *Quambalaria coyrecup* are also being conducted, with its purpose to confirm that *Phytophthora* root infection predisposes marri to canker disease.



Figure 1. Healthy marri tree in full flower and a cankered marri stem.

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Fungi impacting forage production: fungal friend and fungal foe

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The interaction of microorganisms associated with forage species can greatly impact production and longevity of perennial forage crops. The success of cool-season forage grasses (e.g. tall fescue) is attributed to a fungal seed borne symbiont from the genus *Epichloë*. *Epichloë* species represent a dynamic group of endophytes that systemically infect above ground host tissue and vary in transmission strategies (horizontal, vertical or both), reproduction (sexual or asexual), and genome content (nonhybrid or hybrid). These endophytes are capable of producing a range of alkaloids that can protect the host from herbivory but some alkaloids reduce livestock quality. Considerable genotypic and chemotypic diversity exist within the symbiont alkaloid biosynthetic pathways and genome sequencing and genotyping indicate symbiont variation within a host and between host populations. In contrast to the beneficial symbionts, the root pathogen *Phymatotrichopsis omnivora* is considered a natural born killer of dicotyledonous plants, including *Medicago sativa* (alfalfa/lucerne). This pathogen is endemic to southwest USA and will dramatically reduce alfalfa stand productivity. There are no known mechanisms of resistance but aerial imaging is being used to identify areas of stand loss, follow disease progression and determine the cost of production losses caused by *P. omnivora*.

Safeguarding cultural and functional integrity of forests by harmonising cultural and scientific values

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SUMMARY

Disease is expressed when environmental conditions favour pathogens and insect-pests, when tree-hosts are stressed. The Kauri *Agathis australis* (D. Don) Lindl. ex Loudon forests of New Zealand are being compromised by a novel *Phytophthora* pathogen, and the Pine Forests of western-USA by mountain pine bark beetles and a blue-stain fungus. Coastal forests on Fraser Island, a World-Heritage, sand island north of Brisbane, Queensland, Australia, is subject to the effects of strip-mining and water pollution stressors from recreational activities.

All of these systems are subject to the effects of global climate change and environmental pollutants (which predispose trees to diseases) (1), with risks from global plant trade, mineral exploration and development providing avenues for exotic incursions. The focus of the study is to pre-emptively identify the legal, cultural, social and economic impediments which prevent the timely implementation of scientific research to develop incursion response procedures.

The role of 'research' (i.e. quantitative, qualitative, pragmatic and participatory) is to navigate through the constraints to achieve consensus among stakeholders which will allow delivery of 'best practice' solutions to guide the 'sustainable' development of natural and mineral resources. Outcomes will include biosecurity measures to maintain functional, cultural, ecological and economic-values of our forest resources.

A historical overview of the development of the conservation philosophy, leads to an exploration of the 'tragedy of the commons' where we reflect on the current utilitarian approach to nature conservation and the revenue-based, eco-tourism models which could potentially undermine certain 'values' attributed to forests, ranges and wildlands (3).

The tensions that exist between resource development, e.g. timber extraction, recreational usage, mineral and energy development, water catchment, grazing pressure, global food security, and conservation are highlighted in relation to ecosystem services that are being put at risk. These include (but are not limited to) totemic / mythological values, fibre and food production, water and nutrient cycling, carbon sequestration, oxygen production, aesthetic values, land stability, and wildlife habitat.

The 'role' of science and scientific research both in its historical context and in the present day format is then dissected. The provision of solutions to current global problem, need to be undertaken within political and economic environment where funding is restricted and the imperative is for applied solutions (and negative results may never be published).

This leads to a discussion of why empirical science and scientists are marginalised and identifies what barriers exist which prevent up-take of scientific discoveries. The way

forward is to 'go beyond science' to adopt new models of community engagement, participatory decision making, and embracing other forms of research; qualitative, pragmatic, and participatory.

Through the establishment of 'trans-disciplinary' teams (4), proactive engagement of key stakeholders and the input from historians, anthropologists, social scientists educators and qualitative researchers, more socially-relevant outputs can be delivered. The elements of a biosecurity charter are articulated which include; 1) pre-emptively, and pro-actively, engaging key stakeholders, before a response to an incursion is needed, 2) viewing these stakeholders as 'part of the solution', rather than 'part of the problem', and 3) through collaboration and consultation achieve consensus around a set of contingency responses. In this way, we increase ownership and up-take, because we are preserving values that the stakeholders want protected.

Using the model of the Kauri Dieback Joint Agency Response, being undertaken by the central and regional governances of New Zealand, we reflect on how recognising and protecting 'values', both biophysical and cultural, can be achieved through an inclusive, culturally sensitive consultative process. Previously conflicting parties have achieved timely consensus and are actualising biosecurity outcomes to preserve this iconic species for future generations.

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Predicting high-impact pests and pathogens of plants

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ABSTRACT

Prioritisation of pests and pathogens is a key concern for biosecurity agencies worldwide. An important part of this is distinguishing high impact pests from those that cause little or no impact. While there is a large body of research undertaken in pathway analysis as well as pest prioritisation for invasive species, little research in either area has focused on distinguishing high impact from low impact species. Similarly, very little of this research has been applied to produce predictive models to identify high-impact pests and pathogens of plants, for application in regulatory and management situations (unlike invasive plants, which have been researched extensively and for which multiple risk assessment tools have been developed) [1, 2].

We report on the progress of a recently commenced trans-Tasman research project, which aims to predict high-impact pests and pathogens of plants, and invite engagement of plant pathologists with the project by providing expert opinion and assisting with access to difficult to find data sources, often termed 'grey' literature.

Specifically, the project aims to:

- Analyse the historical patterns of plant pest arrivals to Australia and New Zealand, and how these patterns relate to those species which are considered to cause high impacts. The importance of pest biology, pathways, frequency of interception/arrival, and their utility in predicting high-impact pests will be explored.
- Test whether the pathways of high-impact pests differ from those that cause little or no impact. Pathways characteristics such as mode (i.e. ship or plane) and origin of transport, and how they may interact with biological characteristics of high-impact pests will be explored.
- Develop an empirically based and validated prioritisation process for identifying future high-impact pests.

Understanding the drivers of high-impact pest incursions will enable us to test current prioritisation approaches and design a risk assessment framework that will build on those current practices. These drivers may be pathway or pest related, or a product of the interaction of both pathways and pest characteristics.

Ultimately, this project will allow us to test some of the basic assumptions in biosecurity and invasion biology, resulting knowledge to improve prevention and preparedness against future high-impact pests. Basic biosecurity assumptions to be tested include: whether incursion rates for high-impact pests are increasing with time, whether currently monitored pathways are the most likely entry routes for high-impact pest incursions, and whether high-impact pest incursions are largely predictable.

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Human-mediated spread of plant pests via networks

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INTRODUCTION

Traded plant material is suspected to be a significant pathway for the human-mediated movement of plant pests; one that may become increasingly important in the Australasian region as trade continues to rise in the next 10 years. Trade networks, operating at a regional scale, can represent a considerable volume of trade within and between countries, and have been identified as a potential pathway for the spread of diseases. This paper examines the movement of plant pests (specifically nematodes) along fresh produce trade networks in Australasia to determine the risk this pathway poses to Australia as well as to regional biosecurity.

MATERIALS AND METHODS

The recorded distributions of plant-parasitic nematodes (PPN) in the Australasian region were collated to estimate the regional and local species pools. Markets and farms in Australia and several countries in Southeast Asia were surveyed to determine which nematodes were moving through the fresh produce trade networks in this region. Finally, the characteristics of the trade networks were estimated for comparison among countries, and correlated with the frequency and identities of the species that were moving.

Recorded Distribution Data from available surveys as well as published records were compiled into pest lists for each country and used to map the recorded distribution of PPN in the Australasian region. Movement pathways of PPN in this region were also gathered from available interception records.

Nematode Movement Nematodes were extracted from the roots of fresh produce sampled from 27 markets and 23 farms in Australia and several countries in South East Asia to determine which nematodes are moving in this region via this pathway.

Trade Networks Origins of produce and customer information were obtained from questionnaires of subsamples of market traders and consolidated into a single database for each country. The general structure and flow of produce via each trade network were determined using their origin and destination points plotted in Gephi network analysis software.

RESULTS

Recorded Distribution There were 115 PPN recorded from the Australasian region, 36 of these are known agricultural pests and a further 266 are unknown species. The most likely pathways of movement were on or in plants, plant parts and soil.

Nematode Movement Nematodes were extracted from 83% of fresh produce samples. The likelihood of finding a nematode on a market sample ranged from 79-91% in each country surveyed. Plant-parasitic nematodes were found on 53% of samples processed to date. A total of 16 PPN genera were found on crops. Of these, 9 genera were of major economic importance globally.

Trade Networks Each of the trade networks had similar structures and this has implications for the movement of plant pests on them. Perishable crop produce was generally moved relatively short distances between 20-230 km from where it was grown, some produce was moved mid distances between 250-600 km from where it was grown in neighbouring states or provinces, while more storable produce was transported via large scale networks from and to international destinations, between 2,500-12,500 km from the point of origin. Nematodes were found on crops moved locally, between neighbouring states, as well as internationally.

DISCUSSION

This study found nematodes are present in several regional fresh produce trade networks in Australasia and that they have the potential to move through the region via multiple pathways. The information gathered through monitoring of markets can be used to trace outbreaks in real time, not just of nematodes, but fungi, insects and bacteria as well. These data could potentially reduce or prevent outbreaks of nematodes and other plant pathogens that can cause billions of dollars worth of damage to agriculture and industry. Knowledge of the movement of potential pest threats via these pathways may help increase the speed of rapid response efforts as well as reduce the likelihood and size of future outbreaks within and between countries.

Mapping rice diseases for targeted deployment of resistant varieties in India

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INTRODUCTION

The International Rice Research Institute (IRRI), in collaboration with national partners in South Asia, is developing varieties under a project called Stress-Tolerant Rice for Africa and South Asia (STRASA). In the first two phases of STRASA, rice varieties that were adapted to abiotic stresses, specifically drought, salinity, and flooding, were developed and seeds of these varieties were provided to farmers in India. A major effort in the deployment of these varieties is to ensure that seeds are provided to farmers in areas where the adoption of varieties will have the greatest impact. To facilitate the identification of these areas, maps of rice areas in India that are affected by these abiotic stresses were developed.

In this third phase of STRASA, the goal is to distribute 4.6 million tons of seeds of varieties that have genetic resistance to diseases in addition to abiotic stresses. To achieve this goal, the need to identify the areas where these abiotic risks occur with risks of bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae*; brown spot (BS), caused by *Cochliobolus miyabeanus*; and leaf blast (LB), caused by *Magnaporthe oryzae*, was recognised in order to produce integrated maps for biotic and abiotic stresses of rice.

MATERIALS AND METHODS

Daily weather data were extracted from the NASA POWER climatology data set (Stackhouse, 2006) and corrected (Sparks, unpublished) for biases in temperature (Bai *et al.* 2010) for the primary rice-growing season and areas in India. Data were downscaled to 0.25 arc-degrees, 25 km² at the equator, for use with subnational boundaries.

EPIRICE, a generic model for rice diseases created for technology targeting (Savary *et al.* 2012), was used to simulate the intensity of BB, BS, and LB. The model uses the R language (R Core Team, 2015) for a susceptible rice variety given only weather data. Disease intensity was calculated as the average of the area under the disease severity progress curve (AUDPC) from 2001 to 2008 in a raster format.

Administrative areas for India containing district-level boundaries were downloaded as an RData file from www.gadm.org. Using R, average AUDPC values for each district-level polygon were extracted for each disease that was generated by EPIRICE.

Generated maps were presented to national partners during a meeting for validation and improvement of accuracy. Areas that were not accurately represented were identified and improvements to increase the accuracy were discussed. National partners provided expert opinion and data sets based on a national survey for comparison and validation.

RESULTS AND DISCUSSION

AUDPC values were ranked for relative risk rankings: low, moderately low, moderately high, and high. The resulting maps of the relative risk of three diseases closely matched expert opinion and disease survey data. The EPIRICE model underestimated the risk of disease in some areas. These

areas were adjusted accordingly to match expert opinion and survey data. New maps were rechecked with national partners.

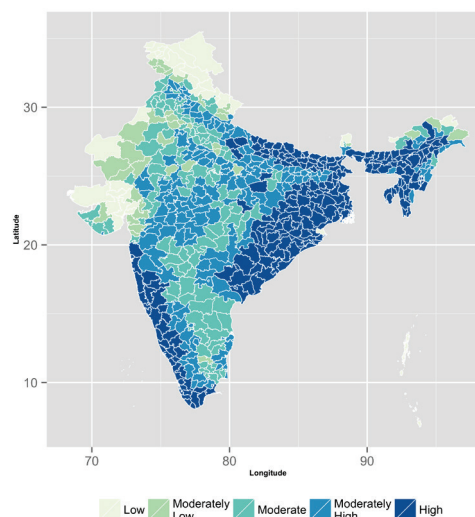


Figure 1. Relative risk of bacterial leaf blight in India based on a susceptible rice variety and selected weather data for the primary growing season from 2001 to 2008. The boundaries and names shown and designations used do not imply official endorsement or acceptance by IRRI.

Once fully completed, the data will be made publicly available. A unified set of data that contains the areas where both biotic and abiotic stresses occur is a novel and valuable tool for identifying areas that are affected by multiple types of stresses.

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Spore trapping methods for the early detection of invasive forest pathogens

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INTRODUCTION

New pathogens in forest ecosystems have been detected at an increasing rate over the past century, primarily as the result of increasing global trade and population mobility. Climate change also threatens well established tree species, making forest ecosystems increasingly vulnerable to disease. The early detection of newly introduced pathogens is a critical component in reducing the impact of new disease threats. This study examines effective aerial spore trapping methods combined with next-generation sequencing (NGS) for the detection of new invasive forest pathogens.

MATERIALS AND METHODS

Two types of active (Burkard-style) trap and one passive (rain gauge) spore trapping method were examined for their effective use in combination with next-generation sequencing (NGS; 454- pyrosequencing). The fungal-specific primers fITS7 and ITS4 were used (Ihrmark et al 2012). Several species-specific PCRs were also undertaken to test the efficacy of NGS (e.g. *Hymenoscyphus pseudoalbidus*, *Dothistroma septosporum* and *Sphaeropsis sapinea*) or to look for pathogens of interest, especially those not amplified by the fungal primers used (e.g. *Phytophthora ramorum*, *Fusarium circinatum*). Traps were located at two forest sites in central and southern Sweden, one high-use port in western Sweden and four forest sites in northern Spain. Trapping was carried out for up to one year at each location.

RESULTS AND DISCUSSION

NGS was successfully carried out on samples from all trap types and from all locations. More than 400 MOTUs of forest fungi were detected including a large number of wood-decay species and at least 100 MOTUs representing forest pathogens.

Different trap types captured different communities of fungi. This could frequently be attributed to the dispersal ecology of the different fungal species. For example, the forest pathogen *Sphaeropsis sapinea* is rain dispersed and was consistently detected in passive (rain gauge) spore traps from Spain but rarely detected from the active traps. In contrast, the root-rot pathogen *Heterobasidion parviporum* has air dispersed spores and was primarily detected in the active spore traps. This clearly indicates the importance of understanding the ecology of the target pathogens when using spore trapping for monitoring purposes.

The fungal-specific primers used in the NGS were not always as accurate as the species-specific primers in detecting certain pathogens. This indicates that where spore trapping is being used to detect particular species of interest, the use of species-specific primers may yield the most accurate results.

Not surprisingly, clear differences in fungal community were found between traps in forest and urban environments. This could be primarily attributed to the presence of fungi associated with different tree species

particular to each environment e.g. *Pinus sylvestris* in forests and *Fraxinus* species in urban environments. This highlights the potential for urban environments such as shipping ports as monitoring sites for new forest pathogens, as forest pathogens would not necessarily naturally be present there and thus be more easily detected when introduced.

Time of year also strongly influenced the fungal communities detected by the spore traps, highlighting the importance of targeted trapping for new invasive pathogens throughout the year.

CONCLUSIONS

This study illustrates the potential for spore trapping and molecular methods to be used as the basis for a comprehensive monitoring network to facilitate early detection of new forest pathogens and help to prevent potentially damaging disease outbreaks. Using NGS, as illustrated here, a broad range of species can be targeted and this is beneficial when new, unexpected or unknown pathogens are present. When designing monitoring systems for forest pathogens using spore traps however it is imperative to consider the limitations of the particular system. This includes identifying potential species of interest and then selecting suitable NGS primers and spore traps and that target these species.

ACKNOWLEDGEMENTS

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Corymbia calophylla (Marri) canker disease incidence at the temporal and landscape level

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INTRODUCTION

Corymbia calophylla (marri), a keystone tree species of southwest of Western Australia (SWWA), is suffering a major decline syndrome associated with the canker fungal pathogen *Quambalaria coyrecup* (1). Anecdotal evidence suggests that the incidence and severity of the disease is increasing, and that remnant stands of marri in anthropogenically disturbed areas are more likely to be affected by canker than forest trees. The current study monitored canker disease progression over a 13-year period at three locations in SWWA. Transects were established at an additional 17 sites to investigate the correlation between anthropogenic disturbance and disease incidence.

MATERIALS AND METHODS

Temporal study In 2001 long term disease monitoring plots were established in three locations with two plots per region; one a disturbed remnant stand of trees and one in adjacent forest. Fifty marri trees were examined for canker presence and ongoing disease progression monitored at each of the six plots, with a final assessment made in 2014.

Disturbance transects 100m by 3m transects were set up at 17 sites across SWWA 1) on road edges adjacent to anthropogenic disturbance, 2) forest road edges, 3) 50m into the forest and 4) 200m into the forest. Marri trees within the transects were assessed for canker presence.

RESULTS

At the time of plot establishment cankers were present on marri at all six sites, with 22.7% of the 300 assessed trees cankered. By 2014 an additional 8.3% of the trees had developed cankers, with death as a result of canker (Figure 1) occurring in 6.7% of trees. Canker incidence was significantly greater on trees present at remnant sites than forest trees from 2001 to 2014 (35.3% increasing to 47.0%, 10.0% increasing to 14.7%, respectively).



Figure 1. Progression of canker development in marri a) 2001, b) 2009 and c) resulting in tree death in 2014.

Marri occurring on road edges adjacent to anthropogenic disturbance (eg farming/grazing) exhibited significantly higher levels of canker disease incidence than marri on a forest road edge. Disease levels continued to drop with

further movement away from the anthropogenic disturbance (Figure 2).

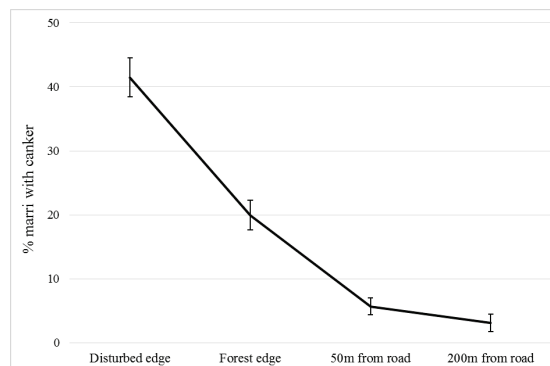


Figure 2. Percent marri with canker, moving from anthropogenically disturbed road edges into forest areas (bars represent standard errors).

DISCUSSION

These surveys provide evidence of the impact this canker disease is having on marri health and its contribution to tree mortality, particularly in areas of anthropogenic disturbance, with worrying implications for the future provision of the valuable ecological, economic and social values these trees provide. Evidence to date suggests that the pathogen is endemic, and further studies are underway to determine the abiotic and biotic factors associated with anthropogenic disturbance that may be driving this relatively recent increase in disease incidence and severity.

ACKNOWLEDGEMENTS

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Rain splash of fungal spores in tree canopies

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INTRODUCTION

Many fungal spores are disseminated via rain. Macro and micro conidia from *Cylindrocarpon heteronema* and unidentified *Arthobotrys*-like spores were released from detached, sporulating apple cankers during simulated rain events to determine spore movement in canopies.

MATERIALS AND METHODS

Experiment 1. Figure 1 describes the spore release approach and set up. There were 4 repeat experiments.

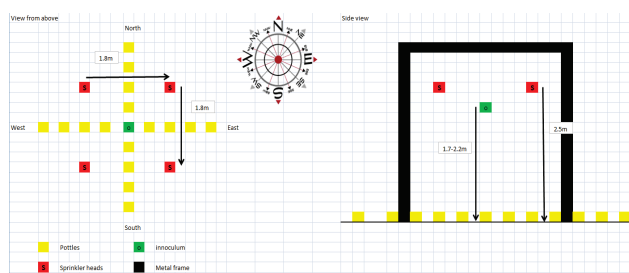


Figure 1. Sporulating cankers were suspended centrally 1.7 m above a sealed surface. The inoculum was surrounded by 4 irrigation sprinklers arranged in a 1.8 m x 1.8 m square 2.5 m above the surface on a metal frame. Simulated rain water and spores were captured in horizontal water traps (250 ml LabServ specimen jars, 65 mm diameter).

Experiment 2. Droplet splash onto two surfaces (sealed and bare soil) was also investigated as illustrated in Figure 2.

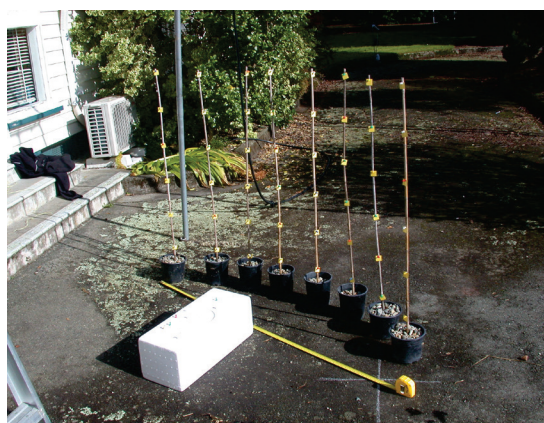


Figure 2. 100 ml of water was dropped in single droplets from various heights. Water sensitive paper pinned onto the bamboo stakes was used to estimate droplet sizes and dispersal (height and distance).

Experiment 3. Droplet splash in a pear tree. A spore suspension was dropped onto a branch of a pear tree from a height of approximately 110 cm and distribution mapped using the methods from Experiment 2.

RESULTS

Experiment 1. Models were fitted where, in the absence of wind, the number of spores (y) decreases with distance (x) according to $y = A \exp(-kx^p)$ where the parameters to be fitted are A = maximum number (directly below the inoculum source, $x=0$) and k, p = shape parameters. Using

this model, we estimated the proportion of inoculum expected to exceed any distance d carried by wind

$$P(\text{spores at distance} > d) = \frac{\int_d^{\infty} x \exp(-kx^p) dx}{\int_0^{\infty} x \exp(-kx^p) dx}$$

For $p=0.5$ and $k=5.7$, this would suggest that even in the absence of wind, about half of all spores end up beyond 0.42 m from the inoculum source, and 4% over 2 m.

Experiment 2. Droplet splash height and distance was similar for the two surfaces. Splash was up to 120 cm outwards and 60 cm upwards. Smaller droplets travelled further than larger droplets.

Experiment 3. At the time of writing data are still being analysed.

DISCUSSION

Water-sensitive paper is an effective method to determine droplet splash and dispersal, allowing measuring droplet size and distance travelled. The non-linear models fitted to spore counts estimated the distance and proportion of inoculum dispersed from a central inoculum source. The model suggested that 50% of all spores splashed beyond 0.42 m from the inoculum source, 4 and 1% travelled over 2 and 3 m, respectively. In the case of *C. heteronema*, 1% of inoculum would be sufficient to cause infections with 50 spores required to infect leaf scars in field situations¹. As expected, smaller spores travelled further than larger spores. In an eradication or disease containment system, the infective propagule size of the rain-dispersed pathogen, point of release and wind parameters during the rain event will determine its distance of spread. This combined with the potential inoculum produced, inoculum concentration required to cause an infection and number of suitable infections sites will determine the zone of eradication and/or area to be monitored. It is hypothesised that for rain-dispersed fungal pathogens in tree canopies the zone will be less than 50 m. We are currently evaluating this.

ACKNOWLEDGEMENTS

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New oomycete genus and hybrid *Phytophthora* discovered as part of stream-based catchment surveillance for kauri dieback

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INTRODUCTION

Kauri dieback, caused by *Phytophthora* 'taxon Agathis' (PTA) has necessitated a national long-term response in New Zealand, as it threatens the biodiversity and cultural values associated with the iconic Kauri *Agathis australis* (D. Don) Lindl. ex Loudon. The disease is present throughout the geographic range of the remnant stands, including parts of the water catchments west of Auckland (1).

Characterisation of the *Phytophthora* diversity in forest-streams have led to the recognition of the common occurrence of *P. gonapodyides* and other member of ITS Clade 6 in waterways and adjacent riparian vegetation (2). Catchment-wide, passive surveillance of PTA commenced in 2009, this included point-sampling, and a year-long study, as part of an MSc-level thesis. The current work 'revisits' two sites from the 2009 study to see if there have been any changes in density and diversity of *Phytophthora* species present.

MATERIALS AND METHODS

Study sites The study area was situated in the Waitakere Ranges, west of Auckland City (GPS: 2649586E; 2S). One site was on the Waitakere River and the other on the Maioha Streams, with each being monitored over two sampling occasions in December 2014 and January 2015.

Baiting apparatus Bait bags were made from re-purposed, 'delicates bags', measuring 30 cm x 40 cm, and constructed from a nylon-polyester mesh with holes of five mm in diameter. These bags were selected as they were light weight, inert, could be sterilised using 5% hypochlorite solution / autoclaved and were re-usable. 'Bubble wrap' was sewn inside each bag along one of the long edges to provide buoyancy. Three different leaf baits were used; *A. australis*, *Pittosporum tenuifolium*, and *Pinus radiata*, with ten baits per bag, and five replicate bags per stream.

Bait processing After either 7 days (if water temperature >15°C) or 14 days, the bait bags were retrieved and returned to the laboratory. Leaf baits were extracted and immersed in 70% ethanol for 2 minutes, and then rinsed in sterile reverse osmosis water. Baits were then dried and plated to *Phytophthora*-selective media. Characteristic gametangial structures of homothallic oomycetes were observed, with pure mycelial cultures being maintained on potato dextrose agar for PCR sequencing.

PCR-sequence analysis All isolates had the ITS2/4 locus sequenced, with ambiguous entities also having β -tubulin amplified from pure mycelial cultures of representative cultures of the various morpho-types identified. Sequences were blasted against NCBI Genbank® to confirm morphological identifications.

RESULTS

Diversity of oomycetes A total of 104 isolates were recovered and sequenced from the two sampling periods, with *Pinus radiata* (36.5%) being the most preferred leaf-bait, followed by *Pittosporum tenuifolium* (34.5%) and kauri

(29%) (n=2). From Sample 1, 56% of the recovered isolates were *Phytophthora* species, while in Sample 2, only 38% of the isolates recovered were *Phytophthora* species (Table 1).

Characteristics of new genus The newly discovered taxa is basal, phylogenetically, to other *Phytophthora* species—with a distant affinity with Clade 9 taxa.

Table 1. Frequency (%) of oomycete taxa recovered from Sample 1 (n=43) and Sample 2 (n=61).

Oomycete	Sample 1	Sample 2
<i>Phytophthora amnicola</i>	16	29
<i>P. amnicola</i> (hybrid)	4	-
<i>P. cinnamomi</i>	-	2
<i>P. citricola</i> s.s	2	-
<i>P. kernoviae</i>	2	-
<i>P. multivora</i>	12	3
<i>P. 'taxon PG chlamydo'</i>	8	2
<i>P. sp. 'Waitakere'</i>	12	2
<i>Phytophthora</i> spp.	30	31
<i>Pythium</i> spp.	14	31

Hybrid with *P. amnicola* We recovered a hybrid-type involving two described parent species; *P. amnicola* / *P. 'taxon PG chlamydo'*. This hybrid entity has been previously collected from waterways in West Australia and South Africa (3).

DISCUSSION

This study demonstrates how leaf baiting can assist to characterise the oomycetes present in forest catchments. This study demonstrated the ability of stream baiting to discover:

- New genera of oomycetes;
- Pathogens of kauri (e.g. *P. cinnamomi*);
- Potential new root rot pathogens with unknown host range (e.g. *P. multivora*);
- Hybrid entities formed between two described, parental *Phytophthora* spp.

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RNA silencing of RNAi effectors reduces root-knot nematode infection

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INTRODUCTION

RNA interference is an endogenous gene regulation mechanism in eukaryotes and has become the most widely used method for functional genomics studies. Root knot nematodes are the most damaging plant parasitic nematodes: current control methods are not fully effective or economically feasible. Gene knockdown experiments in parasitic nematodes involve soaking in complementary double stranded RNA (dsRNA) as well as delivery through plants (1, 2). In this experiment RNAi has been used both *in vitro* and *in planta* to study effects on nematode parasitism by knocking down genes involved in the RNAi pathway.

Four genes involved in the processing of dsRNA and miRNA were studied. Both *in vitro* and *in planta* studies resulted in reduced infection and developmental defects in nematodes subjected to RNAi. RNAi thus presents a potential method to control these damaging pests.

MATERIALS AND METHODS

DsRNA Synthesis: Genes involved in the dsRNA and microRNA processing complex were identified from whole genome sequences of *M. incognita* and primers were designed to amplify a series of potential target genes, which were then cloned into a transcription vector to synthesise dsRNA complementary to the gene (3).

***In vitro* RNAi:** J2 stage nematodes were soaked in a solution containing dsRNA (3). Phenotypes were observed after 16 hours and wild-type tomato plants were infected with dsRNA soaked nematodes to evaluate infection ability by counting galls formed on roots after four weeks.

***In planta* RNAi:** Genes resulting in significantly lower infection after *in vitro* RNAi were cloned into the plant transformation vector (pART27). Arabidopsis ecotype Col-0 was used for transformation by floral dip with transformed *A. tumefaciens* GV3101 (4). T2 plants were infected by nematodes for infection studies. Galls were counted on infected roots after three weeks while nematodes were dissected out of the plant roots after four weeks to study developmental effects.

RESULTS AND DISCUSSION

RNAi phenotypes: DsRNA soaking of J2s resulted in abnormal phenotypes compared to controls. Nematodes soaked in dsRNA complementary to some target genes were straight and paralysed, while those for others were abnormally wavy or paralysed.

Plant infection assessment: Galls per gram dry root weight were calculated to assess nematode infection both after *in vitro* RNAi and as a result of host-induced gene silencing. Four target genes significantly reduced nematode infection with the least infection (90% reduction) observed in nematodes treated with *gene 1* dsRNA (Fig 1). Reduction in nematode infection was in the range of 45-72% for three other target genes.

Similarly, the transgenic events analysed in the study showed a significant reduction in nematode infection. Two events with the least infection for each gene are presented in Fig 2. Up to 89% reduction in infection was observed for

gene 3 plants while reduction in nematode infection was 71% for *gene 1*, 74% for *gene 2* and 69% for *gene 4* transgenic plants.

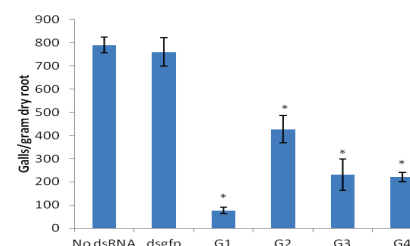


Figure1: Average number of galls/g dry root of plants infected with dsRNA-fed nematodes. G1-4 indicates different target genes. Significance ($p < 0.05$) is represented by *. Bars represent mean ($n=6-7$) $\pm$ standard error.

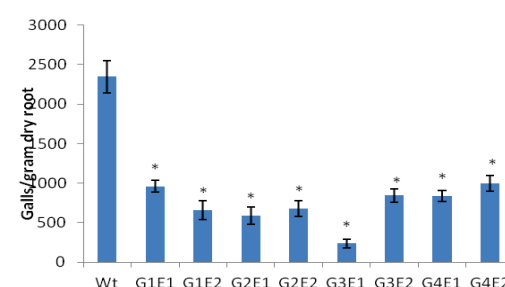


Figure 2: Nematode infection of transgenic plants (galls/g dry root). G1-4 represent 4 target genes, E refers to event number. Significance ($p < 0.05$) is represented by *. Bars represent mean ($n=10-11$) $\pm$ standard error.

Nematodes dissected from transgenic plants had developmental defects including abnormal body shape and transparent appearance. *In vitro* RNAi results did translate into similar infection reduction through host induced gene silencing. The potential resistance shown here can be stacked with plant resistance already present in crop varieties to confer effective nematode control. For obligate parasites such as plant nematodes, RNAi is also a powerful tool for reverse genetics studies, and can be used to probe mechanisms underlying parasitism and development.

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The prevailing pathogen(s) within the black spot disease complex determines expression of host resistance in field pea (*Pisum sativum*)

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INTRODUCTION

Black spot is a devastating disease on field pea caused by a complex of pathogens, the most important of which in Australia include *Didymella pinodes*, *Phoma pinodella* and *Phoma koolunga* (1). Until now there have not been any success in developing high level or complete resistance against black spot in the pea germplasm. Breeding for resistance has had only modest success. In addition, recently another challenge has been raised, that of the large temporal and spatial changes of the population of pathogens involved in the black spot complex in Western Australia (2). This is particularly challenging for breeding programs in Australia and elsewhere that have been largely focused on resistance to *D. pinodes*, ignoring the other pathogens of the black spot complex. Host resistance targeting only one or two pathogens of the complex may shift the make-up of the population toward pathogen species least challenged by the host resistance (2). Therefore, to make resistance more durable, a combination of individual resistances against each of the pathogens in the complex is necessary.

The aim of this study was to determine the relative resistance responses on leaf and stem of 40 different field pea genotypes against three important pathogens associated with black spot, viz. *D. pinodes*, *P. pinodella*, and *P. koolunga*.

MATERIALS AND METHODS

Inoculation: Three week old seedlings of 40 field pea genotypes were inoculated with the three pathogens, viz. *D. pinodes*, *P. koolunga*, and *P. pinodella* under controlled environment conditions. To ensure consistent disease development on both stem and leaf, a new inoculation method was developed, using filter paper discs impregnated with conidial suspension (Fig. 1). After inoculation, seedlings were maintained for three days in plastic boxes covered with plastic sheets. A 0.5cm ca. layer of water was added into the bottom of the box to create high humidity condition.



Figure 1. Black spot symptom on leaf and stem of a susceptible genotype caused by inoculation with filter paper discs impregnated with conidial suspension of *Phoma koolunga*.

Disease assessment: Disease assessment was conducted at 7, 10, 17, and 24 days after inoculation. Scoring categories, 0-7, as proposed by Wroth (3) were employed with some modifications to accommodate the application of conidia-impregnated paper discs for inoculation

Experimental design: Disease severity was calculated as AUDPC (Area Under Disease Progress Curve). A completely randomised design with four replications was used. Each

replication was mean of disease score of the four leaves or two internodes inoculated on one plant. All experiments were fully repeated at least once.

RESULTS

P. koolunga caused the most severe stem disease, significantly more severe than both *D. pinodes* and *P. pinodella*. Against *D. pinodes* and *P. pinodella*, four and three genotypes showed resistance on both stem and leaf, respectively. A few genotypes showing resistance against two and/or three major pathogens of the complex will be of particular important for breeding programs.

Table 1. Response of 40 pea genotypes to inoculation with *Didymella pinodes*, *Phoma pinodella*, and *P. koolunga*.

	No. genotypes showing resistance		
	Stem	Leaf	Both stem and leaf
<i>P. koolunga</i>	5	19	2
<i>D. pinodes</i>	4	16	4
<i>P. pinodella</i>	4	8	3
Two pathogens	8	7	2
All three pathogens	1	1	0

DISCUSSION

Important genotypes with resistance on both leaf and stem and others with leaf or stem resistance against each individual and/or multiple pathogens have been identified in this study.

The difference in response of field pea genotypes to the individual pathogens in the black spot complex reported in this study has added another challenge to the difficulties that pea breeders are already facing, viz. (i) diversity of the pathogens constituting the black spot complex, and (ii) temporal and spatial changes of the relative individual pathogen proportions (2). These challenges make developing field pea varieties resistant to the black spot complex extremely challenging.

ACKNOWLEDGEMENTS

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A new seedling bioassay technique for screening for resistance to white leaf spot disease

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INTRODUCTION

Current management of White Leaf Spot (*Pseudocercospora capsellae*) relies heavily upon chemical control options, but these often only provide partial control and can be cost prohibitive. Effective host resistance to *P. capsellae* offers the best option for white leaf spot to be successfully managed. Therefore, the feasibility of using rapid less expensive, reliable cotyledon screening test that has already been developed for other Brassica pathogens (1) was tested for white leaf spot disease. Differences in resistance across 100 diverse cruciferous genotypes to *P. capsellae* were assessed by field-inoculation of plants with *P. capsellae*. Comparisons for expression of resistance in cotyledons under controlled environmental conditions were made across the same 100 genotypes. Reactions on *Brassica napus*, *B. juncea*, *B. carinata*, *B. oleracea*, *B. campestris*, *B. nigra*, *B. rapa*, *Crambe abyssinica*, *Eruca sativa*, *Eruca vesicaria*, *Raphanus raphanistrum*, *Raphanus sativus*, and *Sinapis arvensis* to this pathogen were tested.

MATERIALS AND METHODS

Field screening at Crawley site. 100 accessions of test genotypes were grown in an experimental field as complete randomised block with seven replicates at the University of Western Australia, Crawley, Western Australia. Plants at the 4-6 leaf stage were spray inoculated with the mixture of mycelial fragments ($4 \times 10^6 \text{ mL}^{-1}$) of four different isolates of *P. capsellae*. White leaf spot disease incidence on each genotype was assessed by separate 9 independent scores made on % leaves diseased from which Area Under Disease Progress Curve (AUDPC) values were calculated for each genotype.

Screening under controlled environment conditions. The same 100 genotypes evaluated for resistance under field conditions were evaluated for seedling resistance under controlled environment conditions. Experiment was a complete randomised block design with four replicates and fully repeated once. All plants were maintained at 15°C, 12 h photoperiod and a light intensity of $380 \mu\text{Em}^{-2}\text{s}^{-1}$. Ten-day-old cotyledons of each seedling were inoculated by depositing a single drop (10 μl) per cotyledon of the mixture of mycelial fragments ($4 \times 10^6 \text{ mL}^{-1}$) of same four different isolates of *P. capsellae*. Disease reactions were recorded at two assessment times, viz. 14 and 21 dpi and the mean lesion diameter for each genotype and % cotyledon disease indices (%CDI) computed.

RESULTS

A high degree of correlation between field and controlled environment experiments was observed (Fig 1).

Further, there was a wide range in host response from high resistance to high susceptibility to white leaf spot. At the field site, *B. carinata* ATC 94129 was the most resistant genotype with AUDPC = 1.2, followed by *C. abyssinica* (AUDPC 8.7), *E. sativa* Eruc-01 (AUDPC 19.3) and *E. vesicaria* Yellow rocket (AUDPC 19.4). In trials under controlled environmental conditions, the most resistant genotype was *B. carinata* ATC 94129 with %CDI values of 0 and 0.2,

respectively, across two experiments, along with *B. napus* genotypes Zhongyou 821 and Hyola 42, with a %CDI value of 0 in one of the two experiments.

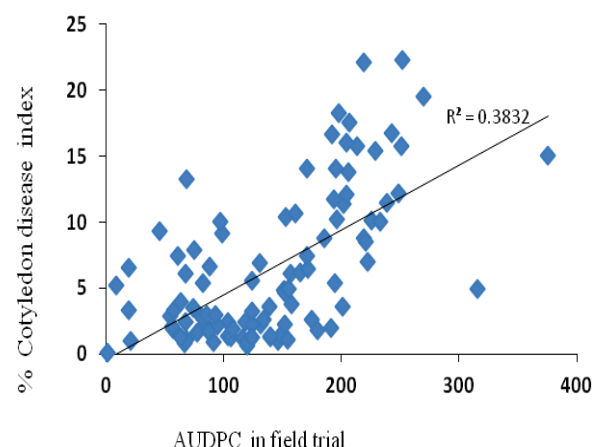


Figure 1. Correlation for genotype responses to *P. capsellae* as measured by AUDPC for percentage leaves diseased and mean %CDI for cotyledon lesion size under two separate controlled environment studies.

DISCUSSION

The disease parameters measured under field conditions to assess the white leaf spot level significantly correlated with %CDI on cotyledons under controlled environmental conditions, highlighting the potential of using rapid cotyledon assays to reliably identify resistance to *P. capsellae*. Our study is the first to demonstrate the applicability of the cotyledon test across diverse crucifer species. Further, as in field screening tests, overall results of controlled environment experiments also illustrated that *B. juncea* genotypes are generally more susceptible to *P. capsellae*.

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Two sides of the coin: investigating defence responses against *Phytophthora cinnamomi* in *Eucalyptus nitens* using dual RNA sequencing

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INTRODUCTION

Damage caused by *Phytophthora cinnamomi* Rands remains an important concern on forest tree species. The pathogen causes root and collar rot, stem cankers and dieback of various economically important *Eucalyptus* spp. In natural *Eucalyptus marginata* forests in Western Australia the pathogen has caused extensive damage to native ecosystems [1, 2] and remains a threat to *Eucalyptus* plantation productivity. In South Africa, susceptible cold tolerant *Eucalyptus* plantations have been affected by various *Phytophthora* spp. with *P. cinnamomi* considered one of the most virulent [3]. Although *Eucalyptus nitens* would be considered resistant to *P. cinnamomi* in its native environment, the species succumbs to the pathogen within forestry plantations in South Africa and isolates of this pathogen were obtained from diseased roots of *E. nitens* [4].

Dual RNA-sequencing allows the concurrent detection of both pathogen and host transcripts during infection. The aim of our study was to determine pathogenicity factors, potential manipulation targets and attempted host defence mechanisms activated by *E. nitens*, contributing to the susceptible outcome of the interaction.

MATERIALS AND METHODS

Year old *E. nitens* cuttings (Sappi Forests Research, Shaw Research Centre, KwaZulu-Natal, South Africa) were stem with *P. cinnamomi* using a 4 mm cork borer and mycelial plug. Mock-inoculated plants were treated similarly except the plug was devoid of pathogen. RNA was harvested from a section of the stem harbouring the inoculation site five days post inoculation and submitted to BGI (Hong Kong) for sequencing. RNA sequencing was performed using an Illumina HiSeq 2000 and 50 bp, paired-end reads were obtained. Reads were separately mapped to the *E. grandis* v1.1 and *P. cinnamomi* var *cinnamomi* (JGI project identity 1003775) genomes and the fragments per kilobase of transcript per million fragments mapped (FPKM) values obtained and differentially expressed genes in *E. Nitens* using programs in the Tuxedo suite [5]. Over-represented Gene Ontologies (GO) in the category Biological Process was determined for the up and down-regulated datasets. Genes or transcripts which showed FPKM values >100 in the 3 inoculated samples with a coefficient of variation < 0.2 were analysed using the pathogen host interactions (PHI) database (<http://www.phi-base.org/>) to determine pathogenicity or virulence factors expressed in *planta*.

RESULTS AND DISCUSSION

Approximately 1% of the reads mapped to the draft genome of *P. cinnamomi* while 78% of the reads mapped to the *E. grandis* genome. One of the most highly expressed genes during infection was a putative crinkler effector (*CRN1*). Phylogenetic analysis indicated the high similarity of this *P. cinnamomi* *CRN1* to that obtained from *P. infestans*. Previous knock-out studies indicated that the *CRN1* ortholog may be involved in suppressing plant defence responses. In *E. nitens*, over 1400 genes were significantly differentially expressed five days post

inoculation in comparison to mock inoculated trees. The gene ontology terms that were over-represented related to hormone signaling and biosynthesis, secondary metabolism and flavonoid biosynthesis and drought stress response. Over-represented GO terms within the down-regulated dataset related to cell wall synthesis, growth hormones and lignin biosynthesis. We observed differential expression of suites of pathogenesis related (*PR*) genes. In particular, a *PR-9* gene, thought to be involved in cross-linking and strengthening cell walls, was two-fold down-regulated in *E. nitens* upon pathogen challenge. This gene shares high similarity to a *Carica papaya* *PR-9* ortholog previously shown to be suppressed upon infection by *P. palmivora* [6]. This *PR-9* gene may thus represent a cross-species effector target during *P. cinnamomi* infection. While we have successfully identified several putative pathogenicity factors expressed in *P. cinnamomi* during infection of *E. nitens* and corresponding responses in the host, comparison to a resistant interaction and further functional experiments will provide support for factors contributing to compatibility.

ACKNOWLEDGEMENTS

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Principals to sustain crop productivity and quality

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Vegetables confer unrivaled profiles of minerals, vitamins and nutrients to enhance public and animal health, incomes and trade opportunities. However, for this potential to be realised, the impact of diseases, both pre and post-harvest, must be carefully controlled in a cost efficient and practical manner. We inhabit a world where globalisation, climate change, increased human mobility, and pathogen and vector evolution have combined to increase the introduction, establishment and spread of plant diseases. The impact of any given pathogen, on the productivity and quality of food produced, is further exacerbated by intensive agricultural production systems and non-durable land use management practices. Some potential solutions will be presented including fit for purpose diagnostics and targeted epidemiology studies, as first steps towards deploying appropriate control interventions. Regional surveillance systems, pest risk analyses and synergy through networks that function to achieve common goals will be discussed. Sustainable and integrated management approaches for plant pathogens will be highlighted that encompass host resistance, agronomic practices and biological control. Food safety issues will be addressed, including mycotoxins, pesticides, heavy metals and transfer of human pathogens via wet markets, and solutions proposed.

Myrtle rust, impacts on Myrtaceous diversity in Australia

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INTRODUCTION

Puccinia psidii was first described from *Psidium guajava* (Guava) in Brazil in 1884 (1), from which its common name *guava rust* was derived. The disease has since been reported from a range of plant species in the Myrtaceae in South and Central America as well as the United States (Florida and California) (1). More recently, *P. psidii* has been reported outside of the Americas, with detections in Hawaii (5), Japan (3), China (6) and South Africa (4).

In 2010, *P. psidii*, myrtle rust, was detected for the first time on the central coast of New South Wales (2), spreading rapidly to be now widespread on the east coast of Australia. Our studies aimed to determine the host range and impact of *P. psidii* on species of environmental and commercial significance.

MATERIALS AND METHODS

Host range and distribution. To determine the host range of *P. psidii* following the initial detection of the disease in Qld, inspections of retail, wholesale and production nurseries were conducted in addition to surveillance in parks, gardens and natural bushland areas.

Symptoms and impact. Targeted surveys were conducted to determine susceptibility of host species to *P. psidii*. These surveys were conducted in public parks and surrounding bushland, private gardens as well as arboreta, National parks, State Forests and reserves in New South Wales and Queensland. Long term monitoring plots have been established at a number of sites with assessments taking place monthly.

RESULTS

Symptoms and impact. Symptoms of infection by *P. psidii* ranged from minor leaf spots to severe foliage and stem blight as well as infection on flowers and fruit of some species. Based on observations to date, 67 host species have been rated as having low susceptibility to *P. psidii*, with only a small percentage of leaves with 1-2 sori per leaf recorded. A further 50 host species were considered moderately susceptible with higher numbers of sori per leaf and a greater percentage of expanding foliage and shoots infected.

Forty-eight species were rated as highly or extremely susceptible with infection occurring on a high percentage of expanding leaves and shoots and evidence of shoot and stem dieback. Highly or extremely susceptible species include the environmentally significant *Melaleuca quinquenervia*, and the rare and endangered species *Backhousia oligantha*, *Gossia gonoclada* and *Rhodamnia angustifolia*.

Severe dieback has been identified on a number of species across their native range including *Decaspermum humile*, *Lenwebbia* sp. Blackall Range, *Melaleuca nodosa*, *Rhodomyrtus psidioides*, *Rhodamnia madeniana*, and *Rhodamnia rubescens*. Tree deaths have been recorded for *R. psidioides* and *R. rubescens*.

Long term impact studies have identified impact on range of species including *M. quinquenervia* where repeated has caused reduced growth rates, altered tree form and reduced flowering rates. Impact on coppice regeneration has also been found to be significant for a number of species.

DISCUSSION

The host range of *P. psidii* in Australia has increased rapidly, with field surveys identifying more than 220 host species from 51 different genera of Myrtaceae. *Puccinia psidii* has now been identified from many different native Australian forest ecosystems including coastal heath, coastal and river wetlands, sand islands, and littoral, montane, subtropical and tropical rainforests. The impact of *P. psidii* on individual trees and shrubs has ranged from minor leaf spots to dieback and reduced fecundity. Tree death as a result of repeated infection has also been recorded for some species, with regenerating seedlings becoming infected and killed by *P. psidii*. *Puccinia psidii* infection has been recorded on flower buds, flowers and fruits of 28 host species. The full impact of this disease in Australia may not be realised for some years.

ACKNOWLEDGEMENTS

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Injection-infiltration of attached grapefruit with *Xanthomonas citri* subsp. *citri* to evaluate seasonal population dynamics in citrus canker lesions

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INTRODUCTION

Asiatic citrus canker is a leaf, stem, and fruit spotting disease of citrus caused by *Xanthomonas citri* subsp. *citri* (Xcc) (3). In subtropical and tropical citrus production areas of the world, citrus canker produces erumpent lesions on susceptible citrus cultivars, especially grapefruit (*Citrus paradisi*). When conducive conditions for bacterial infection prevail, the disease causes defoliation, fruit drop and blemished fruit that have limited or no marketability as fresh produce (2).

The epidemiological potential of harvested fruit to produce inoculum and act as sources for transmission and infection of susceptible hosts has only recently been studied for Satsuma mandarin (*C. reticulata*) in Japan, grapefruit in Florida and lemons (*C. limon*) in Argentina (3). These studies demonstrate that bacteria from fruit lesions have little or no potential for spread to suitable hosts from inoculum sources including infected fruit that have been discarded as packinghouse culls in the field. The conclusion is that harvested and packinghouse-treated citrus fruit are extremely unlikely to be a pathway for Xcc to reach and infect susceptible citrus and become established in canker-free areas (3).

The purpose of this study was to develop a reproducible inoculation method for fruit on the tree so that the population dynamics of Xcc could be assessed as the fruit grow and change in susceptibility to infection. Increased knowledge of Xcc inoculum potential in different age lesions will help to further define the risk that infected fruit pose for transmitting canker bacteria to citrus in canker-free production regions.

MATERIALS AND METHODS

Fruit inoculation and bacterial population assessment.

Two experiments were performed in the 2012 and 2013 growing seasons at the UF-CREC, Lake Alfred, FL. The results for 2013 are presented here. A portable pulse needle-free injector (2) was used to deliver 10 µl of bacterial suspension at 720–790 kPa to infiltrate stomata without causing injury to the fruit rind. Inoculation of attached fruit was necessary because detached fruit even though incubated under ideal temperature and moisture conditions failed to produce lesions. Fruit of different size classes where situated on 20 yr-old 'Ruby Red' grapefruit trees. Inoculations with Xcc at 10^5 cfu/ml were performed on attached fruit varying from 15 to 80 mm in diameter (Table 1). After lesions formed, four to six individual lesions were collected from three separate fruit every 2 wk until mature fruit abscised. Lesions were excised from the rind and macerated in 200 µl of sterile PBS buffer before 5-fold dilution plating on KCB medium (2). Plates were incubated at 28°C for 72 h and bacterial colonies were expressed as log cfu/lesion.

RESULTS

After injection-infiltration of fruit with Xcc, erumpent canker lesions of uniform size formed within 2 wk. Lesions expanded from 1–2 mm to 7–9 mm in diameter on fruit size classes < 55 mm. Inoculation of fruit > 55 mm in diameter, produced lesions but they failed to expand beyond 1–2 mm.

Attempts to inoculate fruit > 80 mm diameter failed to produce lesions after multiple attempts to injection-infiltrate. Populations in lesions on smaller size class fruit were 7–8 log cfu/lesion by July and lesions maintained these population levels up to 6 wk after inoculation through to mid-September (Fig. 1). As fruit colored and the weather became dryer and cooler in November, populations dropped to ~4 log units. Xcc populations continued to decrease with temperature in December to as low as ~3 log units, but resurged to 5 log units after a warm period in February 2014.

Table 1. Size class of 'Ruby Red' grapefruit used for in situ inoculation with Xcc

Fruit dia (mm)	Number of positive inoculation attempts ^z
15–25	4
26–40	2
41–55	2
> 56–70	0

^z 5 fruit per inoculation

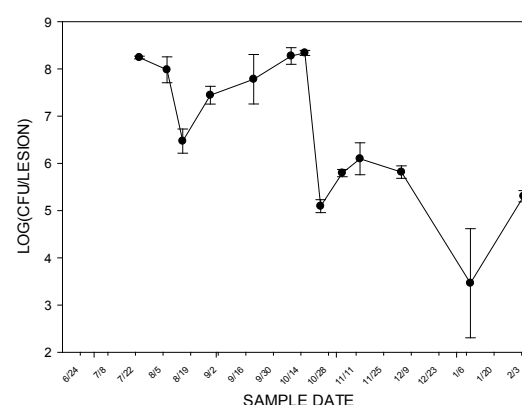


Figure 1. Lesions produced after injection infiltration of 'Ruby Red' grapefruit *in situ* with Xcc at 10^5 cfu/ml

DISCUSSION

Gottwald et al. (3) reported that lesions on symptomatic grapefruit fruit often did not produce detectable Xcc and concluded that mature fruit lesions are an insignificant source of Xcc. In a recent study natural lesions of mixed age and size on mature fruit before harvest were occasionally active (1). Injection-infiltration produced lesions of the similar size and age, as a result, Xcc populations declined more predictably and progressively than populations in naturally occurring lesions (1). As the fruit rind colored and the weather became cooler and dryer October through December, Xcc populations declined to 3 to 4 log cfu/lesion but resurged in January during a period of unseasonably warm temperatures.

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Microbial and microclimatic profiles of almonds during field storage

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INTRODUCTION

Almond nuts are mostly stored in the field either covered or uncovered for several months until they are processed. The nuts may be exposed to the rain, fluctuating temperature and moisture during outdoor storage. *Penicillium* spp., which causes postharvest rots of fruit, can also infect almonds during field storage. However, there has been little study on this pathogen on almonds.

Hull rot of almonds is caused by *Rhizopus* spp. (1). The infection usually begins at hull split, results in blackening of nuts. There is little or no information on the continued growth of the fungus on nuts during storage in the field. More information on the growth of the two fungal species during field storage is needed to improve understanding of postharvest diseases of almonds.

MATERIALS AND METHODS

A trial was conducted to monitor temperature (T) and relative humidity (RH) in uncovered (UC) and covered (COV) almond stockpiles, consisted of 'Carmel' and 'Price' cultivars respectively which are both hard-shelled almonds. Moisture contents of almond kernels, and hulls together with shells were determined after incubating in the oven for three hours at 100°C. The difference in the weight of samples before and after incubation was calculated and presented as % moisture.

Microbial levels on nuts at the beginning of the monitoring period and after nine weeks storage were monitored. Two samples each of 20 nuts from the surface and middle layers of the stockpiles were homogenised, and the extracts plated in duplicate on Potato Dextrose Agar. Colony forming unit per g of nut tissue (CFU/g) were assessed after incubation at 24°C.

RESULTS AND DISCUSSION

Uncovered stockpiles were exposed to the rain on three occasions during the nine weeks (April-June 2014), the T and RH profiles were different from those covered with a tarp (Figure 1).

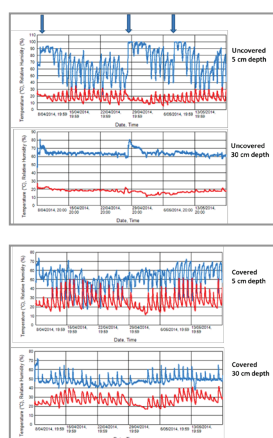


Figure 1. Temperature and relative humidity profiles of uncovered and covered almond stockpiles. RH: ----- Temperature: - - - - - , Rain: ↓

Rain exposure caused higher levels of moisture to be detected in nuts from the surface of the uncovered stockpile than those under cover (Figure 2). The results suggest the practice of not covering nuts may cause nuts to retain higher moisture content, if there is insufficient warmth in the air to help the nuts to dry. The prolonged moisture will then promote growth of microorganisms.

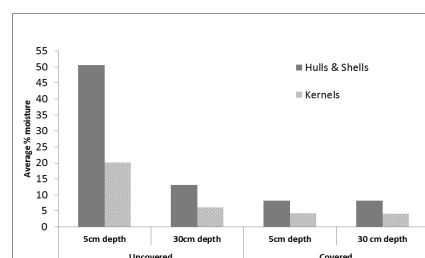


Figure 2: Moisture contents of almond kernels and hulls/shells in covered and uncovered stockpiles.

The numbers of *Rhizopus* spp. in both the covered and uncovered stockpiles increased markedly over the storage period (Figure 3). However, the levels of *Penicillium* spp. declined only with uncovered nuts on the surface of the stockpile. The reason for the decline in *Penicillium* numbers will need to be investigated in future studies.

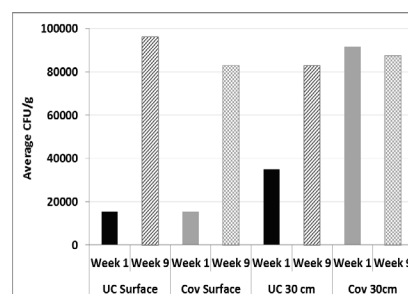


Figure 3: *Rhizopus* numbers in covered and uncovered stockpiles in the first and ninth week of storage.

The results indicate hull rot of almonds which generally is considered to be a disease during the growing season, can continue to develop in storage, and cause further blackening of hulls, shells and kernels. If the sorting machine detects a blemished kernel, a cluster of kernels in the proximity of the blemished kernels will be rejected, resulting in further reduction in the marketable yield. Thus, hull rot has a significant role in causing further downgrade of almond quality and yield postharvest.

ACKNOWLEDGEMENTS

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Studies of sexual reproduction of *Phyllosticta citricarpa*, the causal agent of citrus black spot

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INTRODUCTION

The fungus *Phyllosticta citricarpa* causes citrus black spot (CBS) disease of *Citrus* spp. CBS is one of the most important fungal diseases of *Citrus* (1).

P. citricarpa is known to produce numerous conidia, spermatia and ascospores in nature, but it does not produce pseudothecia in culture (1, 2, 3). Attempts to induce sexual reproduction of *P. citricarpa* *in vitro* have failed, constraining further understanding of the population biology of the species.

In ascomycetous fungi, homothallic species are able to self-fertilise, whereas heterothallic species require participation of an opposite mating type for fertilisation. The mating systems of heterothallic ascomycetes have been shown to be controlled by a single genetic locus (MAT) (4). In some species, spermatia might be involved as gamete cells in fertilisation by fusing to receptive organs of the opposite mating type (1, 5). Although the mechanism of sexual reproduction of *P. citricarpa* is unknown, two mating-type genes (MAT1-1 and MAT1-2) have been identified in *P. citricarpa* isolates (6), supporting the hypothesis that *P. citricarpa* is heterothallic. This study is being performed to (a) determine the presence of different mating types in the Australian *P. citricarpa* population, (b) test the vegetative and sexual compatibility of *P. citricarpa* mating type populations and (c) test if spermatia play a role in sexual reproduction of *P. citricarpa*.

MATERIALS AND METHODS

The mating type locus (MAT) of *P. citricarpa* was characterised by constructing a genome sequence with next-generation sequencing technology (6) at the University of Florida as parts of this collaborated project.

***In vitro* agar mating assay** Isolates of *P. citricarpa* were paired with themselves and isolates of the opposite mating type by placing two parallel mycelial strips (3cm x 2mm) on half strength PDA at a distance of 3 cm from each other. The plates were incubated at 25°C with a light/dark cycle of 12/12 hours for 30 days. The interactions between the pairs of isolates were observed.

***In vitro* leaf disc mating assay** Four autoclaved Myer lemon leaf discs (1 cm in diameter) were placed on moistened sterile filter papers in a petri dish. The discs were inoculated at opposite edges with two mycelial plugs (2mm in diameter) of various combinations of isolates and mating types. Plates were then subjected to different conditions including alternate wetting and drying, various light regimes, and fertilisation strategies with spermatia of the opposite mating types. The leaf discs were examined weekly for the presence of reproductive structures.

***In planta* mating assay** Leaves of sweet orange seedlings were inoculated with suspensions of different combinations of conidia, spermatia and different mating types by soaking blotting papers in the various suspensions and then adhering them to leaves using paper clips. Plants were kept in humid conditions by covering them in plastic bags for 96 hours before removing the bags and blotting papers. Fourteen days after inoculation, leaves were harvested to

be incubated in the laboratory and were subjected to weekly fertilisation with spermatia suspension and alternate wetting and drying conditions. Leaves were examined weekly for the presence of reproductive structures.

RESULTS AND DISCUSSION

The *in vitro* mating assay showed that all of the combinations of isolates remained separated by a zone of growth inhibition between the two colonies. This indicated that *P. citricarpa* isolates were vegetatively incompatible on the culture media, even when paired with themselves. As two compatible nuclei should be brought together in the same cell for sexual reproduction to occur in ascomycetes (4), vegetative incompatibility in the *P. citricarpa* population prevents the opportunity for fusion of genetically different mycelia to initiate sexual reproduction. It is, therefore, likely that sexual reproduction of *P. citricarpa* does not happen via mycelia interaction with other mechanism involved such as spermatisation under appropriate conditions. Results from the ongoing assays would help to prove this hypothesis.

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Temperature adaptation in isolates of *Sclerotinia sclerotiorum* infecting *Brassica carinata*

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INTRODUCTION

Sclerotinia stem rot (SSR) caused by *Sclerotinia sclerotiorum* is a serious disease in oilseed *Brassica* crops world-wide. Reports on the most favourable temperature for development of disease caused by *S. sclerotiorum* vary considerably, with the reported upper limit ranging from 26–30°C, the lower limit ranging from 0–7°C and an optimum temperature a little above 20°C.

The question of whether *S. sclerotiorum* isolates are adapted to the temperature conditions from their region of origin is almost completely unexplored. The only information available comes from a study by Huang and Kozub (1), who found that the best temperature for stimulation of carpogenic germination of sclerotia was 10°C for one isolate, but 25 to 30°C for two other isolates. The current study was initiated to determine whether temperature adaptation could be demonstrated for *S. sclerotiorum* isolates collected from different climatic zones in Western Australia.

MATERIALS AND METHODS

Two isolates of *S. sclerotiorum* collected from infected tissue of *B. napus* were used. MBRS-1 was collected from Mount Barker, and WW3 was collected from Walkaway, where mean annual maximum temperatures are 20.1°C and 25.9°C respectively. Cotyledons of *B. carinata* BC05411344 were inoculated with a mycelial suspension of either isolate, then held at 22/18°C or 28/24°C day/night temperatures. Lesion diameter was compared 44 hours post inoculation (hpi). Stems of 35-day-old *B. carinata* BC054113 (resistant) and SMP3-82 (susceptible) were inoculated with agar plugs containing mycelium, then held at 14/10°C, 18/14°C, 25/21°C or 28/24°C. Lesion length on the stems was compared 72 hpi.

RESULTS

The reaction to temperature of *S. sclerotiorum* isolate WW3 collected from oilseed rape (*B. napus*) in a warmer northern agricultural region of Western Australia differed from isolate MBRS-1 from a cooler southern region. Increasing temperature from 22/18°C to 28/24°C increased lesion diameter on cotyledons of *B. carinata* BC05411344 more than ten-fold for WW3, but did not affect lesion size for MBRS-1. Mean lesion length averaged across two *B. carinata* genotypes (resistant and susceptible) fell from 4.6 mm to 2.4 mm for MBRS-1 when temperature increased from 25/21°C to 28/24°C but rose for WW3 (2.3 mm and 3.2 mm, respectively). WW3, usually designated as low in virulence, caused as much disease on stems at 28/24°C as MBRS-1, historically designated as highly virulent.

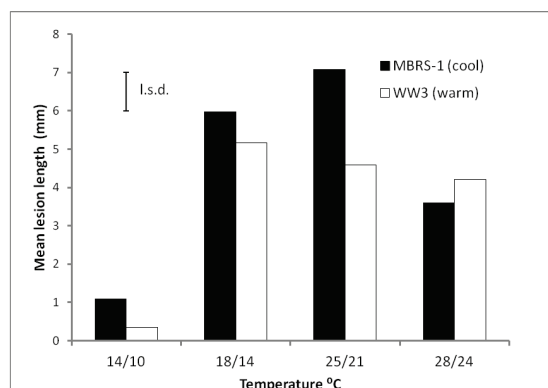


Figure 1. Mean lesion length (mm) 72 hours post inoculation on the stems of 35-day-old *Brassica carinata* SMP3-82 plants inoculated with two isolates of *Sclerotinia sclerotiorum* (MBRS-1 and WW3) at four different temperatures. (l.s.d. is for two way isolate x temperature interaction).

DISCUSSION

This is the first demonstration on any crop of temperature adaptation in isolates of *S. sclerotiorum* collected from differing climatic zones. These findings impact on the methodology used to develop SSR-resistant oilseed *Brassica* cultivars and highlight the importance of screening with disease isolates which are relevant to the area for which breeding is carried out. Characterisation of the temperature adaptation and pathotype (2) of isolates should be carried out before they are used to assess SSR resistance.

ACKNOWLEDGEMENTS

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Teratosphaeria pseudoeucalypti, recently detected and seriously damaging red gum eucalypts in Uruguay

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INTRODUCTION

In 2011, a new and severe blight disease was observed on *Eucalyptus camaldulensis* and *E. tereticornis*, in the eastern part of Uruguay. *Teratosphaeria pseudoeucalypti* was identified as the causal agent of this disease (Soria *et al.*, 2014). The disease incidence has subsequently increased substantially with many trees dead or dying. The aim of this study was to confirm the identify the causal agent of the disease, to identify the host range and to screen for resistance in provenances of *E. camaldulensis* and *E. tereticornis*

MATERIALS AND METHODS

Sampling, culture collection and identification. Sampling was conducted throughout Uruguay from 2012 onwards. Symptomatic leaves were collected from affected plantations, and transported to the laboratory for analysis. Fresh leaves were examined under a microscope and direct isolations were made by lifting conidia oozing from single pycnidia with a sterile needle and transferring these to agar medium. Identification was based on colony s and spore morphology and confirmed based on DNA sequence comparisons for the ITS, Bt and EF1- α gene regions.

Screening for resistance. Susceptibility in *E. camaldulensis* and *E. tereticornis*, and resistance variation amongst *E. tereticornis* provenances was assessed in two field trials naturally inoculated with this pathogen. Genetic material of *E. tereticornis* included 163 open pollinated families from 34 provenances of Australia, Argentina and Uruguay. In both trials, disease severity was assessed 3 years after planting.

RESULTS

The causal organism of the leaf blight disease was identified as *Teratosphaeria pseudoeucalypti*, confirmed based on morphology and multigene analyses, grouping with sequences MUC598 and MUC599 from Andjic *et al.* (2010).

Field surveys showed that the disease, which was first observed in 2011, has now spread throughout Uruguay.

Defoliation in *E. camaldulensis* was higher than in *E. tereticornis*, with an average of 36 and 6%, respectively. Significant genetic variation at population, provenance and family level was found in *E. tereticornis*.

DISCUSSION

The leaf blight disease caused by *T. pseudoeucalypti* has spread throughout Uruguay where it is seriously threatening the most susceptible species (i.e. *E. camaldulensis* and *E. tereticornis*). But it has also been found on *E. dunnii*, *E. globulus*, *E. grandis*, *E. botryoides*, *E. macarthurii*, and hybrids of *E. grandis* x *camaldulensis*. Together with the lerp psyllid (*Glycaspis brimblecombei*), the bronze bug (*Thaumastocoris peregrinus*), and the recently reported blue gum chalcid (*Leptocybe invasa*), leaf blight caused by *T. pseudoeucalypti* adds a severe additional

stress to commercially propagated red gum eucalypts that have performed well in Uruguay in the past.

Our results showed that *E. camaldulensis* is significantly more susceptible than *E. tereticornis*. Furthermore differences in genetic resistance were found amongst *E. tereticornis* provenances. The high susceptibility of some Uruguayan provenances of *E. tereticornis* probably indicates natural hybridisation with *E. camaldulensis*. Resistant provenances of this species should now be selected and deployed to minimise the impact of this disease. Further investigations aimed at better understanding the biology and epidemiology of *T. pseudoeucalypti* are underway, together with studies to evaluate the threat that this pathogen poses to exotic and native Myrtaceous hosts.

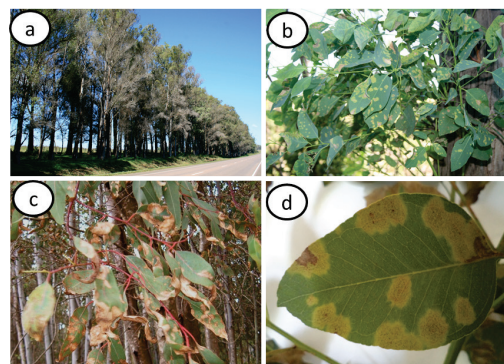


Figure 1. a) Red gum plantation affected by leaf blight disease; b) juvenile leaves of *E. camaldulensis* severely infected; c) leaf blight on adult leaves; d) new lesions on juvenile leaf.

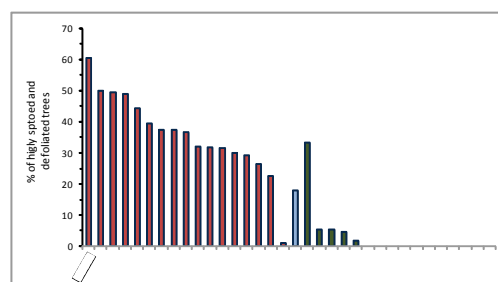


Figure 2. Susceptibility of different provenances of *E. tereticornis* to *T. pseudoeucalypti*. UR = Uruguay; ARG = Argentina; AU = Australia.

ACKNOWLEDGEMENTS

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Plant parasitic nematodes causing strife in Western Australian broadacre crops

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INTRODUCTION

Broadacre cropping generates over \$3 billion for Western Australia's (WA) economy each year with the production of about 10 million tonnes of cereals, oilseeds and pulses. WA's broadacre cropping area of 7 million ha is concentrated in the south-west of the state and more than 65% of paddocks are infested with root lesion nematode (RLN), a major pest of cereal and oilseed crops. It is estimated that RLN populations are at potentially yield limiting levels in at least 40% of infested paddocks. The main species of RLN found in broadacre cropping in WA are *Pratylenchus neglectus*, *P. quasitereoides* (formerly *P. teres*), *P. thornei* and *P. penetrans*, with *P. neglectus* being most common.

The Department of Agriculture and Food, Western Australia (DAFWA) has been supporting WA growers in root lesion nematode research since 1996. Over five hundred crop varieties have been assessed for resistance to these RLN species and 27 replicated field trials have been conducted to assess variety resistance and yield impacts due to RLN infestation.

MATERIALS AND METHODS

Resistance experiments assess the ability of plants to limit nematode reproduction. Multiplication of parasitic nematodes over the term of a glasshouse trial or a growing season is used to measure the resistance of a crop or variety (multiplication of ≤ 1 indicates a variety is resistant, >1 suggests susceptibility as the population has increased). Test plants are compared with known control varieties, which have varied RLN species resistance, and ranked from susceptible to resistant. Due to the inherent variability of nematode populations, a number of replicated experiments are necessary to confidently define a variety's resistance.

In **field experiments**, natural RLN populations are measured in the soil of test plots of different crop varieties at the beginning and end of a growing season. For **glasshouse experiments**, test crop varieties are inoculated with the RLN species under investigation a week after plant germination and grown for 10 weeks in glasshouse conditions. Nematodes are then extracted from the entire roots and counted microscopically to determine total number of nematodes in each pot.

Yield loss trials which evaluate the tolerance of the crop to presence of nematodes have been conducted from 1996 to the present and have evaluated yield impacts in wheat (20 trials) and barley (7 trials) of *P. neglectus* or *P. quasitereoides*. In these trials natural RLN populations in the field, with a single dominant target species, were manipulated via chemical control or management using rotation with crop varieties of differing susceptibility. Differential RLN populations were then exploited to evaluate yield impacts on test varieties.

RESULTS AND DISCUSSION

Resistance experiments conducted at DAFWA since 2003 have tested approximately 275 crop varieties for resistance to *P. neglectus*, 125 for resistance to *P. quasitereoides* and 100 for resistance to *P. penetrans*. Greater detail of

information has been developed in wheat and barley, the most widely grown crops in WA.

Glasshouse trials indicate resistance of crops types differs across RLN species. Of the crops tested, wheat and barley are most susceptible to *P. neglectus* and *P. quasitereoides*. Canola shows intermediate resistance while lupin and field pea are the most resistant. *P. penetrans* is unique in that all broadacre crops tested to date have been susceptible, with lupins and field pea most susceptible.

In field trials wheat and barley support a similar multiplication of *P. neglectus* while barley is more susceptible to *P. quasitereoides* than wheat. Although only a few field trials have included these crops; faba beans, field peas, lupins and oats appear to be the most resistant crop types for *P. neglectus*. Apart from some cereals, field resistance data for most crops has not been established for *P. quasitereoides* or *P. penetrans*.

In glasshouse trials, multiplication of *P. neglectus* and *P. quasitereoides* in canola usually ranks between wheat/barley and lupins, whereas in a field trial conducted in Katanning multiplication of *P. neglectus* on canola was significantly greater than on wheat or barley. It is possible that canola produces a more extensive root system in the field than in pots and this increased root system supports a larger nematode population. Field trials commencing in 2015 aim to further investigate the relative resistance and tolerance of canola.

While wheat, barley, lupins and field peas behave similarly regarding resistance to *P. neglectus* and *P. quasitereoides*, varietal RLN resistance within crops can differ between nematode species. For example, in WA some barley varieties are more susceptible to these RLN species than the most susceptible wheat varieties. This is different to other states where barley is commonly reported as being more resistant than wheat.

Yield loss trials showed significant yield losses in approximately half of trials conducted with losses of up to 34% and 18% due to RLN infestation in wheat and barley crops, respectively. In about half the trials where RLN have been present in populations greater than the level stated as moderate risk, there have been yield losses of more than 5% which represents an annual income loss of \$20/ha in over 2.5 million hectares.

Understanding of *P. neglectus* wheat and barley cultivar resistance is well developed in WA and variety guides carry resistance rankings, however resistance to *P. quasitereoides* and *P. penetrans* are much less understood. Tolerances of varieties are less defined for all RLN species of impacting crops in WA. DAFWA nematology research continues to develop recommendations for cereal tolerance and reliable rotation crops choices for all 3 relevant RLN species.

ACKNOWLEDGEMENTS

We thank WA growers for allowing our trials on their paddocks and DAFWA colleagues for help and expertise. This research is jointly funded by DAFWA and GRDC.

Identification of the first *turnip mosaic virus* resistance gene in *Brassica juncea*

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INTRODUCTION

Brassica juncea is an important oilseed crop in countries such as Australia, China, India, Canada and Russia. Identification of new sources of host resistance to its main pathogens and understanding their genetic basis constitute a key priority. *Turnip mosaic virus* (TuMV) causes one of the major diseases of *Brassica* oilseed crops worldwide. To date, nine TuMV resistance genes—*TuRB01*, *TuRB01b*, *TuRB03*, *TuRB04*, *TuRB05*, *retr01*, *ConTR01*, *TuRBCH01* and *retr02* have been mapped in the *Brassica* A genome of *B. rapa* or *B. napus* (Li *et al.*, 2015). Information on TuMV resistance genes in *B. juncea*, which has both A and B genomes, is lacking. The aim of was to identify the mode of inheritance of the resistance phenotype consisting of systemic hypersensitivity and plant death (+_{ND}) in *B. juncea*.

MATERIALS AND METHODS

Parental cv. Oasis cl which responds to TuMV infection by developing phenotype +_{ND} was crossed with parental cv. JM 06006 which only develops a susceptible phenotype (+). The responses of plants consisting of both parents and F2 progenies derived from them were evaluated after sap inoculation with isolate WA-Ap1 belonging to TuMV pathotype 8. Numbers of plants with characteristic systemic necrotic (hypersensitive) or non-necrotic (susceptible) phenotypes were recorded at 0–11 days after infection (dai) (early infection) and 12–21 dai (late infection). The Chi-square test was used to determine their fit to a 3:1 ratio (systemic necrotic: non necrotic) with early infection, and a 1:2:1 ratio (+_{ND}:+_N:+) with late infection. Here, +_N refers to systemic infection with necrosis but without plant death.

RESULTS AND DISCUSSION

When the numbers of plants that developed each phenotype were compared, they were in agreement with a 3:1 ratio for necrosis (resistance): lack of necrosis (susceptibility) with early assessments (Table 1). However, for late assessments only a third of the plants with systemic necrosis were subsequently killed (+_{ND} phenotype), the remainder remaining alive (+_N phenotype), giving a final segregation ratio of 1:2:1 for phenotypes +_{ND}:+_N:+ (Table 2). These results suggested presence of a single hypersensitivity gene which was fully dominant at the early stage of infection, but exhibited only partial dominance later. Thus, presence of two dominant alleles (homozygous condition) resulted in phenotype +_{ND}, but presence of only one dominant allele (heterozygous condition) resulted in phenotype+_N. When two recessive alleles were present (recessive homozygous condition), only phenotype + developed. We proposed the name *TuRBJU 01* (TuMV resistance in *Brassica juncea* 01) for the resistance gene (Nyalugwe *et al.* 2015). This follows the same nomenclature (*TuRB01-05*) previously used for TuMV resistance genes in *B. napus* and *B. rapa*. Such systemic hypersensitivity genes have been used for many years by plant breeders. They are effective as infected plants die quickly thus removing them as internal sources of infection for acquisition and further spread by vectors or contact. This reduces the rate of epidemic development resulting in much decreased yield

losses (e.g. Jones *et al.* 2003). It is unknown if *TuRBJU 01* is located on the *B. juncea* A or B genomes.

Table 1. Segregation data for F2 progeny plants with early infection.

		TuMV			
		Number of			
Test	Total F2 plants	Necrotic	Non necrotic	χ^2	<i>P</i>
1	39	28	11	0.21	0.65
2	58	44	14	0.02	0.89
3	112	82	30	0.19	0.66
4	119	90	29	0.03	0.86
Total	328	244	84	0.07	0.79

Table 2. Segregation data for F2 progeny plants with late infection.

Test	Total F2 plants	TuMV			χ^2	P
		Number of		Non necrotic		
		+ _{ND}	+ _N			
1	39	7	21	11	1.5	0.59
2	58	19	25	14	1.97	0.37
3	112	23	61	28	0.19	0.66
4	119	24	67	28	2.16	0.34
Total	328	73	174	81	1.61	0.45

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Assessing resistance and tolerance of cereal varieties to root lesion nematodes in multi-environment field trials

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INTRODUCTION

In the southern region *Pratylenchus neglectus* and *Pratylenchus thornei* are the two most important root lesion nematodes (RLN) affecting broadacre crops. Information on how new varieties affect RLN population densities (resistance) and their ability to yield in presence of significant population densities (tolerance) is important to assist growers when planning their cropping programs.

Resistance classifications have traditionally been determined using glasshouse data. In this paper we investigate use of data from field trials established to evaluate new varieties for tolerance to RLN, to support resistance classifications and explore possible additional benefits to growers this data may provide by using a range of initial population densities.

MATERIALS AND METHODS

Sites and Trial Designs. The field trials were located in SA and Vic between 2011 and 2014 (Table 1). These were established using a split-plot trial design in which adjacent plots (or blocks) of high and low RLN densities were created by growing resistant and susceptible varieties in the previous year.

Measuring Nematode Densities. RLN population densities were assessed in each plot at seeding and post harvest. At each time, a single soil sample was collected that was a composite of 20 cores per plot taken to a depth of 10 cm.

The soil samples were delivered to the SARDI Molecular Diagnostic Centre where DNA was extracted and RLN population density was assessed using species specific TaqMan MGB assays.

Statistical Analysis. A linear mixed multi-environment trial (MET) analysis of the log e-transformed numbers was performed. Residual maximum likelihood was used for variance parameter estimation and BLUPs were produced for multiplication and yield for each entry at each density level within each site.

Covariates were included to account for differences in initial RLN levels between plots. A factor analytic (FA) approach was used to model the genetic correlation between the high and low densities within and across sites. The FA approach allowed for heterogeneity of genetic variance and correlation between the RLN densities within and across sites.

RESULTS AND DISCUSSION

Split Plot Design In the past, nematicides were used to create contrasting nematode densities to assess nematode tolerance in field trials. Many of the products used have been withdrawn from the market. The split plot design in which susceptible and resistant varieties are sown in the previous season achieves a similar result without the OHS risks. This system also enables valuable information for growers to be collected on how each variety affects different nematode population densities.

Linear mixed MET Analysis of Resistance The rankings of varieties for resistance to RLN were generally highly correlated between field trials even though they had different initial RLN densities and were conducted in different states and seasons (Table 1). However, the predicted RLN multiplication rates differed for genotypes between environments at the same initial nematode density.

Analysis of the tolerance data is continuing; preliminary results show stronger GxE effects than for resistance rankings, possibly driven by spring rainfall.

Current investigations are focussing on using MET analyses to try to identify the key factors driving the variation in multiplication rates and yield responses between sites and seasons for both *P. neglectus* and *P. thornei*. It is expected these analyses will simplify the comparison of new varieties with those more familiar to growers in the southern region.

ACKNOWLEDGEMENTS

This project was funded by the GRDC projects DAS00116, DAV123 and DAN00124.

Table 1. High genetic correlation of cereal varietal resistant rankings between SA and VIC trial sites in different seasons.

<i>P. neglectus</i>			2012Pinery	2012Horsham	2013Mallala	2013Horsham	2014Mallala	2014Horsham	2014Wynarka
2012Pinery	SA	1							
2012Horsham	VIC	0.93	1						
2013Mallala	SA	0.84	0.91	1					
2013Horsham	VIC	0.72	0.78	0.77	1				
2014Mallala	SA	0.85	0.93	0.86	0.76	1			
2014Horsham	VIC	0.72	0.65	0.71	0.72	0.66	1		
2014Wynarka	SA	0.84	0.96	0.83	0.67	0.87	0.41	1	
<i>P. thornei</i>			2011Minnipa	2012Banyena	2012Streaky Bay	2013Banyena	2014Banyena	2014Streaky Bay	
2011Minnipa	SA	1							
2012Banyena	VIC	0.89	1						
2012Streaky Bay	SA	0.83	0.95	1					
2013Banyena	VIC	0.98	0.96	0.93	1				
2014Banyena	VIC	0.88	0.98	0.91	0.94	1			
2014Streaky Bay	SA	0.94	0.82	0.87	0.94	0.79	1		

Transcriptional regulation in a maize population segregating for susceptibility to grey leaf spot disease

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INTRODUCTION

Cercospora zeina causes grey leaf spot (GLS), a yield-limiting disease on maize. The objective was to exploit gene expression data from a segregating maize population to dissect the susceptible response to *C. zeina* infection. The project addressed the hypothesis that there are underlying DNA polymorphisms in maize lines that give rise to changes in gene expression, which in turn affect GLS disease severity.

MATERIALS AND METHODS

Agilent 44K maize microarrays were used for global gene expression profiling of earleaf samples collected from 100 recombinant inbred sub-tropical maize lines exposed in the field to *C. zeina*.

RESULTS AND DISCUSSION

Two complementary approaches were used to analyse the gene expression data, namely weighted gene co-expression network analysis and expression QTL analysis. Integration of the data allowed us to explore the genetic basis for coordinated expression responses to GLS disease in susceptible maize plants. These results provide a platform for systems genetics analysis of GLS responses in maize and extend the current knowledge of disease susceptibility in crop plants.

An efficient and reliable glasshouse screening method to evaluate bacterial blight resistance in field pea (*Pisum sativum*)

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INTRODUCTION

Bacterial blight, caused by *Pseudomonas syringae* pathovar *pisi* and/or *P. syringae* pv. *syringae*, is an important disease of field pea (*Pisum sativum*) in many parts of the world. Environmental factors, such as hail and frost, cause conducive conditions that support epidemics in susceptible crops (Richardson and Hollaway 2011). As chemical control of the disease is not effective, the development of field pea cultivars with bacterial blight resistance is a high priority in the breeding program. To enable breeding of resistant germplasm, a high-throughput and reliable seedling assay was developed. The method uses spray inoculation of seedlings in small pots with a bacterial suspension followed by incubation in a glasshouse. This method is less laborious compared to previous stab-inoculation methods, and takes less than one month (c.a. 25-27 days) from seed sowing to disease assessment. The method developed is being used to evaluate bacterial blight resistance within the Australian field pea breeding program and enabled to identify potentially novel sources of resistance to *P. syringae* pvs *pisi* and *syringae*

MATERIALS AND METHODS

Plant material. Seeds of different field pea genotypes were obtained from the Australian Grain Gene bank (AGG) or the Pulse Breeding Australia field pea breeding program, both based at the DEDJTR, Horsham, Victoria

Bacterial inoculum preparation, plant inoculation, and disease assessment. Bacterial suspensions were made in Nutrient Yeast Broth for 10-12 hrs and cells were suspended in tap water. For spray inoculation, the cell concentration was diluted using optical density (OD₆₀₀) measurements. Disease assessment as done on individual plants and converted into the no-parametric scale of 0-9.

RESULTS AND DISCUSSION

In the field, *P. syringae* pathovars *syringae* and *pisi* produced indistinguishable symptoms and are characterised by the appearance of water-soaked lesions, which gradually become olive green, brown and necrotic. The glasshouse screening method showed similar symptoms after infection with both pathovars (Fig. 1). The new screening method is less laborious and appeared to be more reliable than previous methods, such as the stab inoculation process (Hollaway and Bretag 1995). This will facilitate the development of cultivars with disease resistance more quickly.



A B

Fig. 1 Symptoms of bacterial blight infection in field pea. A. In field, B. In Glasshouse

Correlation of the glasshouse method with field performance.

Disease severity ratings developed using the new glasshouse method were highly correlated with those from the field when using a mixed bacterial suspension for inoculation with correlation coefficient of 0.82 (Spearman's rank). Correlations were highly significant ($p < 0.001$) confirming that the glasshouse seedling method is a good predictor of bacterial blight resistance in adult plants under field conditions.

Identification of new sources of resistance to bacterial blight. Large scale screen of landraces germplasm against *P. syringae* pv. *syringae* and pv. *pisi* race 6 identified novel resistant sources. Number of accessions were identified that showed levels of resistance comparable to PBA Percy, the most resistant currently grown cultivar to both pathovars suggesting resistance is complex and partial. (Table 1) These new resistant germplasm will be useful in pyramiding and improving resistances in the breeding program.

Table 1. Disease resistance screening of field pea landraces and wild-types for bacterial blight caused by *P. syringae* pv. *syringae* and pv. *pisi* Race 6

Accessions	P. syringae pv. Syringae		P. syringae pv. pisi Race 6	
	Mean rank		Mean rank	
	(K-W ANOVA) ^a		(K-W ANOVA) ^a	
AGG1498	22.0		ATC1498	59.12
PBA Percy	43.09		ATC2553	59.71
AGG4985	49.69		ATC4984	63.81
AGG4622	62.19		ATC4900	68.50
AGG4900	74.04		ATC4983	73.15
AGG4984	105.15		ATC4391	101.19
PBA Oura	107.42		ATC4622	105.88
AGG4030	112.81		PBA PERCY	110.22

^aDisease response for genotypes were analysed using a non-parametric analysis method, Kruskal-Wallis one-way ANOVA.

Comparisons shown are with resistant cultivar susceptible cultivar Kaspa and PBA Percy, respectively. Significant differences are shown as * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$ and n.s. = non-significant

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The emergence and evolutionary dynamics of maize streak virus

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Maize streak viruses are monot-infecting circular single stranded DNA viruses that belong to the *Mastrevirus* genus in the *Geminiviridae* family. Maize streak virus strain A (MSV-A) is the causal agent of maize streak disease, one of the most serious biotic threats to food security in Africa. Studies into origin, trans-continental spread and changes in its pathogenicity in maize following the spread can yield some valuable insights into the emergence of this pathogen. Additionally, determining where the extant MSV-A lineages arose can help identify geographical hot-spots of MSV evolution. Our model-based phylogeographic analysis of a large dataset of MSV-A full genomes sampled across the continent to reconstruct the plausible history of MSV-A over the past 150 years shows its emergence in Southern Africa in the mid 1800s. Following this, the virus spread trans-continently at an average of ~32.5km / year (95% highest probability density interval, 15.6 – 51.6km/year). Further, our analyses indicates that whereas MSV lineages have been progressively evolving to produce more extensive chlorotic lesions on maize leaves, they have, surprisingly, been concomitantly evolving to induce less pronounced stunting.

Multi-locus genome screening of *Candidatus Liberibacter solanacearum* samples indicates limited genetic diversity of the invasive bacterium within New Zealand

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INTRODUCTION

Candidatus Liberibacter solanacearum (CLso), the putative causal agent of Zebra Chip (ZC) disease of potatoes, is vectored by and associated with *Bactericera cockerelli*, the tomato potato psyllid (TPP). The first report of ZC and CLso in New Zealand was in 2008 (1), not long after the discovery of TPP in 2006 (2). CLso has five described variants (haplotypes): A and B infect solanaceous plants and are reported from the Americas (A and B) and New Zealand (A only), whilst C, D and E infect apiaceous plants (carrot and celery) and are reported from Scandinavia and Europe.

The pathology and symptoms of ZC in New Zealand are different from, and often less severe than symptoms in the USA: nevertheless the disease and insect have caused substantial economic damage in New Zealand, with the incursions costing the potato industry alone NZD120M between 2006 and 2011. There are a range of possibilities for why ZC is less severe in New Zealand than the USA, including potato genotypes, cultivation practices and environmental conditions. In Texas, CLso B has been observed to be more destructive than CLso A: however, genetic variability has been reported in both (3 and references within). Using a recently developed multi-locus sequence typing assay (3), the genetic diversity of CLso A in New Zealand samples was assessed to understand whether potential pathogen genetic variation may contribute to the observed differences in pathology.

MATERIALS AND METHODS

DNA extracts from 27 CLso-positive samples from different years (2009–2014), five solanaceous plant hosts (potato, tomato, thornapple, Jerusalem cherry), and adult and nymph psyllid specimens from throughout New Zealand (Northland, Auckland/Pukekohe, Hawke's Bay, Manawatu, Marlborough and Canterbury) were analysed for the presence of the D348, D406 and D426 genomic loci using the qPCR MLST assay (3). These three loci were selected from the wider MLST set based on distribution within 20 North and Central American samples containing CLso A (3). The D426 locus was present in all American samples, D348 and D406 were the most variable of the loci evaluated being present in 17 and 18 of the 20 samples, respectively (3). All selected CLso samples had previously tested positive for CLso by a semi-nested qPCR assay (4).

RESULTS AND DISCUSSION

TPP nymph or adult specimens collected from four solanaceous plants, as well as plant samples from potato, tomato and tamarillo from throughout New Zealand, which had previously assayed positive for CLso using a semi-nested qPCR assay (4), consistently showed the presence of all three MLST loci. It is important to note that the presence of CLso in psyllids collected from host plants does not infer the presence of CLso in those plants. Various potential alternate solanaceous plant hosts for TPP have been assayed previously for CLso, without any findings of the bacterium. Some samples did not amplify any of the MLST loci: these samples also had very low CLso titres based on

the original 16S qPCR C_T values (>34.5). CLso has three copies of the rRNA operon: this apparent failure to amplify the unique MLST loci reflects the relative abundance of targets in the bacterial genome.

The D348 and D406 loci were the most variable in distribution within CLso A samples from the Americas. These two loci, along with D426, were present in all New Zealand samples, suggesting limited genetic diversity is present in the bacterial populations present in New Zealand. The origin of the TPP present in New Zealand is believed to be the western USA, based on mitochondrial sequence, and it probably entered New Zealand on smuggled plant material (5). The origin of CLso is less clear, although it is generally believed to have arrived with the insect (i.e. not independently in a plant host). Unfortunately, no western USA CLso A samples were available to screen with these MLST assays, and thus provide data potentially to support the hypothesis that the bacterium and the insect have a similar, western USA, geographic origin. The limited genetic diversity of CLso A in New Zealand suggests either a limited number of incursions, or origins from a limited geographic region in the Americas.

ACKNOWLEDGEMENTS

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Can plants tell you if they are infected with viruses?

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INTRODUCTION

Virus infections in plants pose a major threat to agricultural, environmental and economic security globally. Strict monitoring of viruses and other infectious microorganisms that may come with plant materials is implemented as part of border biosecurity by a number of Australasian nations. However, the available detection tools are limited, particularly with regards plant virus detection. Current assays are limited to symptom observations, which may be inaccurate; and molecular tests to detect virus particles or nucleic acids, which require some prior knowledge about the virus.

Rather than detecting specific viruses, this study proposes a potential tool to detect virus infection in plants by looking at host cellular responses to viruses. The study explored molecular indicators of virus infection which include measurement of the amount of low molecular weight RNA and transcript accumulation of evolutionarily conserved stress- and pathogen- responsive genes, using *Arabidopsis thaliana* for proof of concept and validated in two crop plants.

MATERIALS AND METHODS

Arabidopsis thaliana (col-0) plants were infected with five common plant viruses *Tobacco mosaic virus* (TMV), *Cauliflower mosaic virus* (CaMV), *Tomato spotted wilt virus* (TSWV), *Turnip mosaic virus* (TuMV) and *Turnip yellow mosaic virus* (TYMV). Leaf samples were taken at 2, 3, 7, 14, 21, 28 and 35 dpi and total RNA was isolated from three biological replicates of virus- and mock-inoculated plants for each time point. Drought, fungal and bacterial infection experiments were also performed to determine plant responses that are unique to virus infection and not characteristic of other stress and pathogen responses. Experiments were undertaken in kiwifruit and rice to confirm initial findings.

The amount of low molecular weight RNA (LMW-RNA) were quantified using the Bioanalyzer method (Agilent Technologies) while the transcript accumulation of selected stress- and pathogen-responsive genes were quantified using Reverse Transcriptase quantitative PCR (RT-qPCR) (Roche Light Cycler 480, Roche Diagnostics Ltd.). Normalisation was performed with GeNorm using the reference genes FBOX, SAND and EF1 alpha which have been previously validated as good reference genes for virus infection in *A. thaliana* (1).

RESULTS AND DISCUSSION

The amount of LMW-RNA appeared to be correlated with virus infection. Furthermore, out of the 15 candidate genes tested, some genes were found to show significant transcript accumulation change in response to virus. These genes were selected and further analysed in response to drought, fungal and bacterial infections in *Arabidopsis*, kiwifruit and rice.

This study proposes a methodology and formula as an initial tool to determine if plant samples are infected by a virus (Figure 1). This allows for a quick detection of virus-infected samples before testing for the presence of specific viruses if required. The methodology was first tested in *Arabidopsis* and validated in rice and kiwifruit. Further refinement to establish that the method is widely effective includes the development of degenerate primers that are sensitive and specific so as to target corresponding genes in all target plant species of interest.

This method may prove to be a useful tool to link pathogenicity with presence of virus as detected by next generation sequencing.

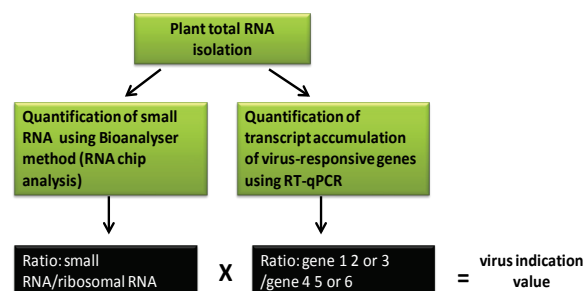


Figure 1. Proposed methodology to determine virus infection in a plant using host molecular indicators.

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Uromycladium acaciae is the cause of severe rust symptoms on *Acacia mearnsii* in South Africa

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INTRODUCTION

A severe outbreak of an unknown rust fungus on *Acacia mearnsii* in the KwaZulu-Natal Province (KZN), South Africa, appeared in 2013. Based on initial microscopic investigation of the teliospores, the rust was identified as a species of *Uromycladium*. It differed in morphology to *U. alpinum*, which was reported from South Africa in 1988 (1).

Surveys were conducted to determine the extent of the disease. We used morphology and DNA sequence comparisons to determine the cause of the rust epidemic on *A. mearnsii* in South Africa.

METHODS AND RESULTS

Specimen collection and symptoms. Rust samples were collected from the KwaZulu-Natal, Limpopo, Mpumalanga and Western Cape Provinces of South Africa, as well as from neighbouring Swaziland. Dried herbarium material from the original collection of *U. alpinum* on *A. mearnsii* in 1988 (1) was obtained for comparative purposes.

Symptoms observed in KZN included leaf and bark spots, gummosis from leaves and stems, defoliation and die-back of young seedlings (Fig. 1). Only isolated/mild leaf spot symptoms were seen in the Limpopo and Western Cape Provinces. Rare gummosis symptoms were observed in Mpumalanga.

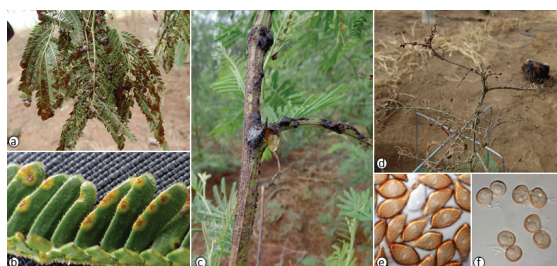


Figure 1. Rust symptoms and spores. (a) gummosis from leaves, (b) uredinia, (c) bark spots and gummosis, (d) defoliated seedling, (e) urediniospores, (f) teliospores.

Phylogenetic analyses. The internal transcribed spacer (ITS) and large subunit (LSU) regions of ribosomal DNA (rDNA) were sequenced for 12 samples from *A. mearnsii*. These sequences were aligned with other species of *Uromycladium* on *Acacia* from Australia. Maximum likelihood (ML) and Bayesian inference (BI), implemented in RAxML and MrBayes, were used to determine the identity of the rust with a phylogenetic approach.

Congruent topologies were obtained from ML and BI (Fig. 2). The rust on *A. mearnsii* in South Africa and *U. acaciae* from Australia is conspecific.

Morphology. Urediniospores and teliospores from different provinces of South Africa were compared. These samples were also compared to the urediniospores on samples from the first report of *U. alpinum* in 1988.

There were no differences in morphology of urediniospores between the rust first collected in 1988 (1) and the current outbreaks on *A. mearnsii*. Telia, with two teliospores per pedicel, lacking hygroscopic cysts, are present on recent collections. The morphology of the teliospores was closest to those of the description of *U. acaciae*, first reported on *A. dealbata*.

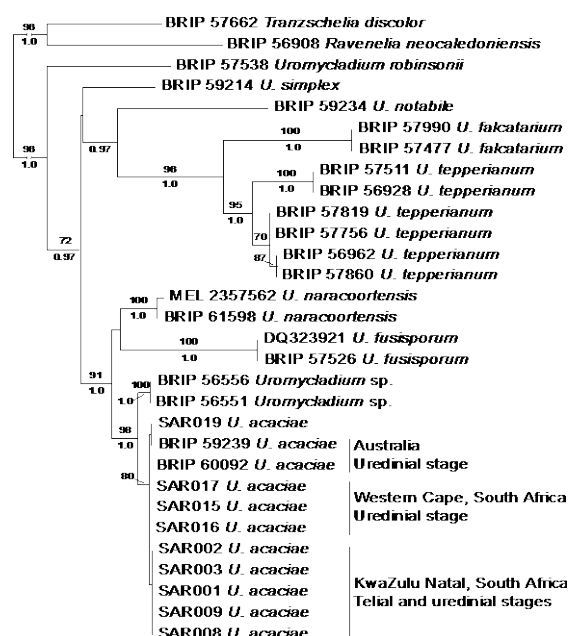


Figure 2. ITS and LSU phylogram from a ML search in RAxML. Bootstrap values (≥70%) from 1000 ML replicates above nodes. Posterior probability values (≥0.95) summarised from 30,004 trees in a Bayesian search below nodes.

CONCLUSIONS

Uromycladium acaciae is the rust on *A. mearnsii* in South Africa and it has been present since 1988. It unexpectedly produced its telial stage, which has since caused death of seedlings, stunting, and die-back. Future investigations will determine why the telial stage was produced and whether this might be linked to weather conditions.

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Characterisation of *Xanthomonas* spp. associated with bacterial leaf spot disease of solanaceous crops

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INTRODUCTION

The *Solanaceae* is a large plant family of about 100 genera and 2,500 species. It is an economically significant family, containing many of the world's important crops, such as potato, eggplant, tobacco, tomato, capsicum and chilli. These plant species and the diseases that affect them have been the focus of much study. A particularly important disease is bacterial leaf spot (BLS), which is a major pathogen of capsicum, chilli and tomato. Although BLS is often very destructive, there has been comparatively little work done on the causal organism.

The causal agents of BLS around the world are currently recognised as a species complex that consists of *Xanthomonas vesicatoria*, *X. euvesicatoria*, *X. gardneri* and *X. perforans* (1). Previously this species complex was classified as *Xanthomonas campestris* pv. *vesicatoria*. Symptoms of BLS include lesions on leaves, stems and fruit which can lead to defoliation, reduced yield or direct fruit damage in tomato, capsicum and chilli. Lesions on stems and leaves are initially small (< 1mm), tan to black, and irregular. The lesions generally become angular (2-5mm), water-soaked, or coalesce to form larger necrotic areas. Lesions on fruit are often raised. Severe infections can result in extensive damage to crops and significant yield losses.

BLS has been reported in Australia, and is common in all tomato growing regions. The distribution of *xanthomonas* species associated with BLS in Australia is uncertain, as all records refer to *X. campestris* pv. *vesicatoria*. Isolates collected in past and current seasons have been used in this study in order to characterise the pathogens causing BLS in Australian tomato, chilli and capsicum crops.

MATERIALS AND METHODS

Biochemical characterisation. Isolates were initially characterised using their biochemical properties. These include oxidase reaction, catalase reaction, KOH gram test, non-fluorescence on KB (King's medium B), production of yellow colonies on YDC (yeast dextrose chalk agar), starch hydrolysis, pectin hydrolysis, and oxidative growth.

Tobacco plants were inoculated with 3 ml of inoculum (0.1 OD) to determine if the isolate produced a hypersensitive reaction after 24–48 hr.

Molecular characterisation. Isolates were identified to species by PCR using specific primers (2). These primers are specific for the four known BLS species, *X. vesicatoria* (Bs-XvF, Bs-XvR), *X. euvesicatoria* (Bs-XeF, Bs-XeR), *X. perforans* (Bs-XpF, Bs-XpR) and *X. gardneri* (Bs-XgF, Bs-XgR).

Isolation and inoculum preparation. Pathogens were cultured from streaming lesions placed in a drop of sterile water. This was then streaked onto nutrient agar and incubated at room temperature. After 2-3 days, a colony of the dominant isolate was sub-cultured for further testing.

Inoculum was prepared from cultures in nutrient broth grown overnight, centrifuged at 8000 rpm for 10 min, and resuspended with sterile distilled water. Suspensions were adjusted to 0.1OD using a BioDrop spectrophotometer.

RESULTS

More than 80 isolates distributed over season, location and host were characterised. A summary of the species associated with certain host crops is detailed in table 1. Many isolates were not PCR positive for any of the four species, though their biochemistry indicates they may be a pathogenic *Xanthomonas* species.

Table 1. *Xanthomonas* spp. collected from Australian crops

Isolates	Host	PCR result
9	Tomato	<i>X. vesicatoria</i>
10	Capsicum	<i>X. euvesicatoria</i>
23	Chilli	<i>X. euvesicatoria</i>
39	Various	Negative

DISCUSSION

Initial findings indicate that there is host specificity within the species of *Xanthomonas* that cause BLS. This is consistent with published literature on race / species specificity of *Xanthomonas* from hosts of BLS. The biochemical properties of the isolates support the PCR results and indicate a high degree of genetic variation. Race testing on differential lines also supports the phenotypic and genetic variation observed in previous experiments and in the literature. The variation and distribution of *Xanthomonas* spp. that cause BLS in Australia will be discussed.

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Fusarium fujikuroi associated with bakanae disease of rice in southern Lao PDR

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INTRODUCTION

Bakanae disease of rice, caused by *Fusarium fujikuroi* has long been known in Asia. It is characterised by elongation of seedlings, and foot rot symptoms may be present in some plants. Foot rot may also be associated with stunted plants. Although bakanae disease has been reported in the Lao PDR (1), to the best of our knowledge, the etiology has not been studied in detail nor has the biology of the pathogen been investigated. The following study was undertaken to compare the situation of bakanae in the Lao PDR with that in Italy (2), where the disease is causing concern in modern rice cultivars.

METHODS AND RESULTS

Samples of rice plants, showing typical symptoms of bakanae disease were collected in 2013 and 2014 from three provinces in southern Laos: Champasak, Salavan and Savannakhet. Symptomatic plants were scattered but easy to detect due to the abnormal stem elongation. However, there were some stunted plants with symptoms of stem base browning and necrosis and these were also collected. The presence of white-violet mycelium was occasionally observed on the stem base of diseased plants. Up to 20 plants were collected from each sampling site. Isolations were attempted by plating a 2 cm length, including the 2nd node, from one symptomatic stem of each plant. Two hundred and seventeen colonies were isolated. Monosporic cultures were grown on CLA and were morphologically identified. Ninety five percent of the fungal isolates recovered were *F. fujikuroi*. A total of 12 populations, ranging from 9 to 24 isolates, were selected. In order to confirm the morphological identification, DNA was extracted from these isolates and a species-specific PCP was conducted on each with primers *FujiIF* (5'ACGTGTCAAACAACTAAACAnCGA-3') and *TEFIR* (5'-GCGACAACATACCAATGACG-3'). Mating type distribution within each population was assessed by PCR using primer pairs M1C-5/M1C-13 and M2C-9/ M2C-4; specific for MAT-1 and MAT-2 alleles, respectively. These results will be presented in the poster.

DISCUSSION

The etiology of bakanae disease in Laos has not been clearly defined to the best of our knowledge, despite it being reported. Through our studies we have clearly demonstrated that *F. fujikuroi* is the dominant *Fusarium* species associated with bakanae disease in southern Laos. The disease is not considered a serious problem in the Lao PDR and was usually found more commonly in traditional cultivars that farmers prefer for home consumption. It was not common in modern higher-yielding rice cultivars, except where the small-holder farmers had been using their own seed of such cultivars for some years. Furthermore, bakanae disease was not observed in the Thasano Seed Multiplication and Research Centre in Savannakhet that selects and produces seed of new cultivars for the southern region. Thus, the situation in the Lao PDR differs from Italy

where the disease is causing concern in modern rice cultivars (2).

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Prevalence and economic impact of parasitic nematodes of field crops in western Victoria

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INTRODUCTION

To set research priorities, and to inform on farm disease management strategies, accurate information on the economic losses caused by plant pathogens is required. The pathogenic root lesion, (*Pratylenchus neglectus* and *P. thornei*) and cereal cyst nematodes, (*Heterodera avenae*) are important in Victoria (3), with yield loss related to the pre-sowing nematode density (1). Less is known about the root lesion (*P. penetrans*) or the stem (*Ditylenchus dipsaci*) nematodes in Victoria.

The widespread use of qPCR (PreDicta B diagnostic service) provides a useful tool for the rapid and accurate estimation of the prevalence and density of soilborne pathogens. This, data when combined with current knowledge on yield losses (1, 2), allows for an estimation of the economic losses caused by these nematodes in Victorian wheat crops.

MATERIALS AND METHODS

Nematode density data from Victorian paddocks was combined with yield loss data from Victorian field experiments to estimate the economic loss from parasitic nematodes. Results from 386 paddocks tested by qPCR (PreDicta B) during 2014 and 2015 were used to determine the prevalence and density of *P. neglectus*, *P. thornei*, *P. penetrans*, *H. avenae* and *D. dipsaci* in the Mallee, Wimmera and Western District regions of Victoria. Grain yield losses were determined from replicated field experiments comparing grain yields of wheat varieties sown in contrasting nematode densities across growing seasons (1, 2). The economic estimations were based on the assumptions that;

- >20 *P. neglectus*/g soil = 2-7% yield loss (2),
- the *P. thornei* densities 5-20, 20-60 and >60 nematodes / g soil, were associated with yield losses of 0-8, 0-15 and 0-20%, respectively (1)
- 75% of wheat varieties grown were intolerant,
- of Victoria's wheat production, 50% was in the Mallee, 24% in the Wimmera and 11% in the Western District,
- Victoria produces 3.7 million tons of wheat per annum,
- the wheat price is AUD\$240 per ton.

RESULTS AND DISCUSSION

The most prevalent nematodes were *P. neglectus* and *P. thornei* (Table 1). The relative density of these two nematodes is shown in Table 2. More than half of the paddocks in the Mallee and Wimmera regions were infested with greater than five *P. neglectus* per gram of soil, with fewer paddocks infested with *P. thornei*. (Table 2). *H. avenae* was observed in 12% of paddocks within the Wimmera and 5% of paddocks in the Mallee demonstrating the success of resistant cultivars (3). *P. penetrans* was rare and *D. dipsaci* was not detected.

In the three regions studied the annual losses due to *P. neglectus* was estimated at \$2.0 to 7.1 million and for *P. thornei* was \$0 to 6.3 million, depending on season. Even

though yield losses due to *P. neglectus* were lower, a higher economic loss was estimated, due to its higher prevalence. Further field trials into yield loss caused by these nematodes are occurring and will enable improved estimation of nematode impacts.

Table 1. The prevalence of five species of *Pratylenchus*, *Heterodera* and *Ditylenchus* nematodes in three grain growing regions of Victoria during 2014 and 2015.

Nematode	Prevalence (%)		
	Mallee	Wimmera	Western District
<i>n</i>	173	182	30
<i>P. neglectus</i>	98	95	63
<i>P. thornei</i>	27	53	27
<i>P. penetrans</i>	0	1	0
<i>H. avenae</i>	5	12	0
<i>D. dipsaci</i>	0	0	0

Table 2. The proportion (%) of paddocks within each nematode density category.

Density Categories Nem/ g soil	Prevalence (%)		
	Mallee	Wimmera	Western District
<i>P. neglectus</i>			
Below detection	2	5	37
0.1-5	33	29	50
5-20	44	47	10
20-60	18	19	3
>60	3	0	0
<i>P. thornei</i>			
Below detection	73	47	73
0.1-5	16	35	24
5-20	10	11	3
20-60	0	6	0
>60	1	1	0

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Managing on-farm biosecurity risk through pre-emptive breeding: the case of rust on field pea and lentil

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INTRODUCTION

Rust is a significant yield constraint in the production of lentil and field pea in various regions of the world. It is the most important foliar disease of lentil in North and East Africa, South America and South Asia. Rust of pea is an important disease in tropical and subtropical areas such as northern India and central China; it also has significant impact in the pea production in temperate regions (e.g. Spain and Canada). In Australia, the causal agents of rust on lentil and field pea (*Uromyces viciae-fabae* and *U. pisi*) have not been detected and therefore they are considered as an exotic plant pathogen (EPP) and a serious threat to the industry.

An international collaborative project was set up with the aim of providing to the lentil and field pea industries the tools to mount a rapid and effective response to the incursion of exotic plant pathogens causing rust. The pre-emptive measures include: the evaluation of current elite cultivars and breeding lines in key rust host-spots, the identification of novel sources of resistance and introgression of resistant loci into locally adapted Australian elite germplasm, and importantly, the assessment of populations' structure of pathogens and their virulence shifts to account for difference between regions. Here we provide an update on evaluation of germplasm in response to rust infection under field and controlled conditions.

MATERIALS AND METHODS

Germplasm evaluation: Germplasm was evaluated in response to rust under field conditions in Ethiopia (two sites subjected to natural infection) and in Spain (artificial inoculation), and under controlled conditions in response to infection with isolates from various origins. Disease severity (%) and infection types (IT=0-4) were recorded in all experiments.

Isolate collection: Commercial and trial sites of Ethiopia, Spain and India were sampled to evaluate the distribution and genetic structure of pathogen populations.

RESULTS AND DISCUSSION

Lentil germplasm response to rust differs from site to site - a likely reflection of the population genetics of the causal agents. At Chefe Donsa (Ethiopia), most lentil Australian breeding lines and cultivars were resistant to the natural infection showing low levels of disease severity (<3%); a wide range of IT were observed and a large proportion showed pustule development (IT=3-4). At this site, most Ethiopian landraces were susceptible to rust infection; among the resistant entries are the cultivars recently released in Ethiopia by ICARDA. Most of the Australian genotypes showed substantially more disease at the second Ethiopian site, Sinana, with scores differing from those in Chefe Donsa.

Lentil and field pea accessions (n > 200) were also evaluated under field and controlled conditions in response to

inoculation with individual isolates of *U. viciae-fabae* and *U. pisi*). Seventeen percent of Australian field pea accessions show disease severity 1-10% upon infection in the field with a single *U. pisi* isolate (Fig. 1; mature pustules (IT=4) were observed in all the accessions. Among the *Lens* accessions, a high proportion (75%) of lines show disease severity about 40% or higher (Fig. 2). Disease severity levels were higher than those observed in Chefe Donsa. Resistant accessions will be evaluated further under controlled conditions in response to a range of isolates and at the field trials in Ethiopia and India under natural infection.

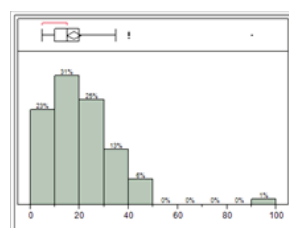


Figure 1. Distribution of field pea accessions according to the disease severity (%) upon infection with *U. pisi* isolate Up-Co01 under field conditions. Above is the outlier and quantile box plot.

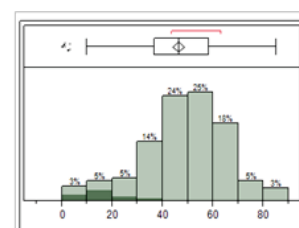


Figure 2. Distribution of lentil accessions according to the disease severity (%) upon infection with *U. viciae-fabae* (ex. lentil) isolate, Uvf-Va10 under field conditions. In dark green, the wild accessions.

Isolates were collected from all field trials and from national surveys to validate results under controlled conditions and to determine the species/subspecies and population composition of the causal agent of rust in lentil and field pea in hot-spot disease areas.

ACKNOWLEDGEMENTS

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Diagnosing rice grassy stunt virus in Indonesia using RT-lamp assays

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INTRODUCTION

Rice Grassy Stunt Virus (RGSV) is an RNA tenuivirus that causes one of the major diseases of rice which is endemic in Indonesia yet exotic to Australia. This economically important disease is listed on the Rice Industry Biosecurity Plan as a High Priority Pest and on the Northern Australia Quarantine Strategy (NAQS) 'Target list of plant pathogens'.

The host range of RGSV includes *Oryza* spp. *Cynodon dactylon*, *Cyperus rotundus*, *Echinochloa colonum*, *Leersia hexandra* and *Monocoria vaginalis*, although not all hosts show clear disease symptoms when infected (1). There is relatively little current information published on RGSV and some confusion in the literature over strains and subspecies.

This disease is vectored by the brown planthopper *Nilaparvata lugens*, which is capable of long distance migratory flights and is present in Australia. The proximity of this disease in Indonesia to northern Australia's expanding rice industry and vast areas of native rice presents a significant biosecurity risk. This project aimed to investigate and diagnose RGSV and other rice viruses in Indonesia using a LAMP assay with the Genie II.

MATERIALS AND METHODS

Survey. Samples were collected from Java Indonesia October 2014. Symptoms, location and host type were recorded before the samples were desiccated over calcium chloride for storage.

Molecular assay—RT-LAMP. Reverse transcription loop-mediated isothermal amplification assays (RT-LAMP) were conducted with the Genie[®] II and lyophilised isothermal master mix (OptiGene[™] Ltd.), Primers (2) and a simple quick DNA extraction (3).

Molecular assay—PCR. The RNA was extracted (QIAGEN RNeasy[®] plant mini kit) then cDNA synthesised (SuperScript III) then amplified by PCR (PCR beads GE Healthcare illustra[™]) with primers (2). The product was amplified and visualised by gel electrophoreses against a molecular marker.

RESULTS AND DISCUSSION

The LAMP assay was able to clearly and reliably diagnose RGSV samples as positive or negative. Results were verified by PCR with key samples verified by sequencing. All marginal LAMP results were confirmed to be negative by PCR. The LAMP assay reduced the diagnosis of RGSV from 2 days to less than 1 hour.

In the field a range of symptoms and severity were seen for RGSV including excessive tillering, stunting and shortened narrowed pale green/yellow leaves. At some farms in Java the whole field was infected resulting in total crop loss, yet at other sites the disease distribution was patchy and only isolated plants were affected.

Many of the samples collected during the survey were infected with two viruses, RGSV and *Rice Ragged Stunt Virus* (RRSV) an RNA oryzavirus. RGSV was detected in

plants as a single infection but RRSV was always detected with RGSV. Both of these viruses are vectored by the same brown planthopper *N. lugens*. There is relatively little information published on RRSV and no information published on the implications of dual infections. Several negative PCR/LAMP results were recorded for samples with symptoms of RRSV possibly indicating confusion over symptoms or presence of another virus or PCR/LAMP RRSV primers not able to detect all variants of the virus.

The disease caused by RGSV appears to be causing more losses in Indonesia in recent years due to increasing chemical resistance in the vector *N. lugens* (S. Hartono personal comm, unpublished). Further research is required to understand these diseases and the nature of the risk to Australian commercial and native rice populations. Of concern is that no National Diagnostic Protocol exists for this disease in Australia should an incursion occur.

ACKNOWLEDGEMENTS

Subcommittee on Plant Health Diagnostics for funding this laboratory residential: Department of Plant Protection, Gadjah Mada University (GMU), Yogyakarta, Java, Indonesia for hosting the laboratory residential; GeneWorks[™] for the loan of the Genie II; and the International Plant Health Programme, Department of Agriculture for use of import permit.

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New *Tiarosporella* species isolated from Australian wheat

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INTRODUCTION

Wheat grain from farm storages across Australia was surveyed for the presence of fungal pathogens over two seasons. The aim of this study was to describe one of the species that was isolated, using morphological and molecular techniques.

MATERIALS AND METHODS

Collection and isolation of fungi. Grains were sampled from farms across five different grain-growing regions of Australia (WA, NSW, QLD, VIC and SA). Fungi were isolated by direct plating and from moisture chamber incubation.

DNA isolation, amplification and sequencing. Mycelia were collected by scraping from the agar surface with a sterile blade and placed in a 1.5 mL sterile microfuge tube and frozen in liquid nitrogen, ground to a fine powder and genomic DNA extracted using a hexadecyl trimethyl ammonium bromide (CTAB) protocol from (2) modified by the addition of 100 µg/ml Proteinase K and 100 µg/ml RNase A to the extraction buffer (3). The DNA of the ITS region was sequenced and data compared to known sequences in Gen bank.

Radial growth rates. Pure cultures of each isolate were grown on full-strength potato dextrose agar (PDA), malt extract (MEA) and corn meal agar (CMA). Plates were incubated for six days at seven different temperatures (4°C, 10°C, 15°C, 20°C, 25°C, 32.5°C and 37.5°C) with the radial growth measured daily.

RESULTS

A new species of *Tiarosporella* was isolated from wheat grain collected from a farm storage in Western Australia.

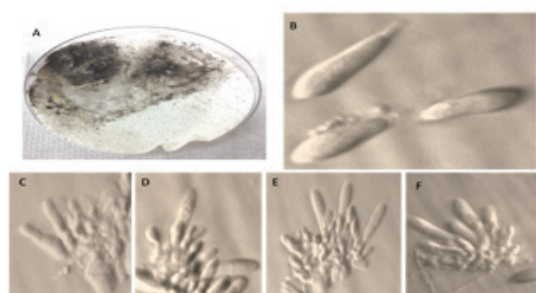


Figure 1. Asexual spores of *Tiarosporella*. A. Pycnidia formed in moist chamber culture on wheat; B. Conidia; C-F Branched conidiophore with conidiogenous cells producing conidia. Bar = 20 µm.

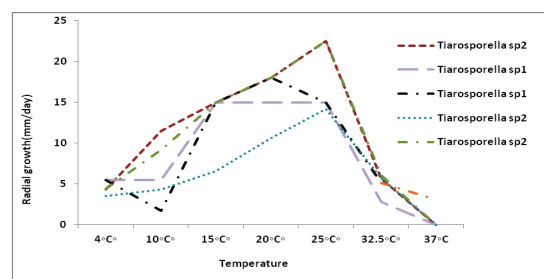


Figure 2. Mean radial growth (mm/day) rates of five isolates of *Tiarosporella* species on potato dextrose agar.

DISCUSSION

This study presents the first report of a putative new *Tiarosporella* species on stored wheat grain in Western Australia. Thomas and Jayasena (2013) reported that *Tiarosporella* spp. affect the quality of wheat grain and cause white grain disease in the field, however did not provide a species name.

Tiarosporella belongs to the family Botryosphaeriaceae which contains species that can cause extensive damage to grain in the field and in storage. It is possible that *Tiarosporella* can cause further damage postharvest if the temperature and moisture content during storage are conducive to fungal growth.

ACKNOWLEDGEMENTS

The first author is grateful to Murdoch University and the Libyan Government for financial support.

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South-west Australia: a global hotspot of virus diversity

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INTRODUCTION

In 2006 scientists first reported using high-throughput sequencing (HTS) of DNA to study the metagenomes of microbial communities. Three years later, virologists began adopting the technology to identify novel virus genomes from plant samples. Since then there has been an explosion in the number of new virus species discovered.

The viruses of indigenous plants in Australia and globally have been sparsely sampled for viruses. In southern Western Australia there exists an unusually diverse range of native plant species. The region is a UN recognised 'hotspot' of species biodiversity. This term is used to refer to the macrobiotic diversity; almost nothing is known about the microbial or viral flora. We decided to begin addressing this deficiency by sampling the plant viral flora of the southwest corner of WA to determine if its diversity mirrors that of the larger organisms indigenous to the region.

MATERIALS AND METHODS

We devised methods for sampling large numbers of wild plants and fungi chosen at random from natural systems, and to identify all of the RNA viruses present using HTS.

RESULTS

A large number of viruses were identified from diverse wild plant hosts. In some cases, subsequent matching of the host and virus enabled isolation and systemic infection of viruses in experimental hosts, but in other cases experimental systemic hosts were not found. The latter situation made it difficult to convince the International Committee on Taxonomy of Viruses to accept the newly identified viruses as genuine, especially if complete genome sequences were not available. Some of the viruses found have roots that link them with globally distributed groups, while others are so far known only from WA.

DISCUSSION

We discuss the importance of southern Western Australia for virus evolution, and implications of virus discoveries for conservation, food production, and biosecurity.

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Wind spread of plant viral pathogens into northern Australia

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INTRODUCTION

Plant virus pathogens pose an increasing biosecurity challenge globally. The most economically important plant viral pathogens occur in tropical and subtropical parts of the world, such as southeast Asia. Potyviruses, luteoviruses, tospoviruses and begomoviruses are the virus groups that cause the most devastating crop diseases, resulting in major quality and yield losses in plant produce worldwide (1). The hypothesis being tested in this study is that economically important plant viral pathogens are arriving in Australia from our northern neighbours via wind-borne insect virus vectors blown across the sea. Previous studies in the Ord River Irrigation Area (ORIA) provided an example of this. Sequence analysis of aphid-borne *Zucchini yellow mosaic virus* (ZYMV) isolates revealed that this is the only place in Australia where the virulent Southeast Asian ZYMV strain occurs (2). This strain is believed to have arrived in the ORIA via viruliferous aphids carried over the sea on wind currents.

Application of viral genomics offers exciting prospects for studying the connectivity between populations of the same plant viruses present in Indonesia/East Timor/Papua New Guinea and Northern Australia. However, few plant viral or insect vector studies are occurring in these three neighbouring countries. Next Generation Sequencing (NGS) offers an ideal way to compare the genomic sequences of viruses found in samples from the same crops collected in different regions. This project will use NGS and focus on potyviruses, *Luteoviridae*, tospoviruses, begomoviruses and mastreviruses. It will aim to improve Australia's biosecurity tactics and responses through establishing the extent and significance of viruses with wind-borne insect vectors arriving from nearby countries and establishing in Northern Australia.

MATERIALS AND METHODS

Sample collections: We will sample crop plants showing virus-like symptoms to obtain whole genome sequences from Indonesia and East Timor as well as from Northern Australia. Leaf samples will be collected from plants with virus-like symptoms of the crops common bean, capsicum, tomato, cucurbits, maize, sugar cane, sweet potato, maize and banana. Leaf samples from Indonesia/East Timor will be preserved on FTA cards before dispatch to Australia. Leaf samples from within Australia will be placed in labelled plastic bags inside cooler boxes and air-freighted to Perth.

Molecular/genomics: With the samples from Indonesia and East Timor, virus nucleic acid will be extracted from FTA cards. With the Australian samples, they will be extracted from leaves. DNA and RNA extraction will be done using Plant Qiagen Mini prep Kits according to the manufacturer instructions. RNA will be used to detect RNA viruses, and DNA to detect DNA viruses. High quality nucleic acid extracts will be subjected to PCR (DNA) or RT-PCR (RNA) using specific and degenerate primers for relevant viruses. For whole genome sequencing, selected infected samples

will be subjected to Nextera and the Truseqstranded Ribozero library preparation according to the Illumina protocol.

Biological studies: With the Australian samples, tissue from virus-infected leaf samples will be tested by ELISA (using relevant virus antisera), and PCR or RT-PCR (using relevant primers). Extracts from positive Australian samples will be inoculated to standard indicator hosts to check for diagnostic virus symptoms. Virus presence in the inoculated plants will be determined by further tests.

With selected viruses of biosecurity concern, to establish Koch's postulates, sap from virus cultures growing in appropriate plant hosts will be inoculated to plants of the crop host they potentially threaten. The inoculated crop plants will be examined for characteristic symptoms and assessed for sensitivity to the virus. Koch's postulates will be established when the disease is reproduced and the virus recovered again. To help determine likely economic impacts on northern Australian crops, additional experiments will involve comparing virus-inoculated and mock-inoculated plants. Some of these experiments will be used to measure dry weights (biomass) and others left to quantify seed losses.

EXPECTED OUTCOMES

The project will provide important information on the connectivity between crop viruses found in northern Australia and Indonesia/East Timor. It will also help gauge the likelihood of virus introduction by viruliferous insect vectors arriving in Australia via natural wind dispersal. The findings from this work are expected to enhance identification of potential plant virus biosecurity threats, improve plant biosecurity threat monitoring research for the north of Australia and, in turn, enhance the biosecurity protection status of Australian agriculture.

ACKNOWLEDGEMENTS

We thank project collaborators in East Timor and Indonesia and AQIS-NARCS staff for help in collecting virus and vector samples, the CRC for Plant Biosecurity for funding this research and the University of Western Australia for a SIRF PhD scholarship.

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Physiology of Sauvignon blanc vines, with and without *Grapevine leafroll-associated virus 3*, over three seasons in Marlborough, New Zealand

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INTRODUCTION

Typically *Grapevine leafroll-associated virus 3* (GLRaV-3) researchers report one or more of the following impacts on vine growth and fruit production in red grapes: reduced photosynthesis, phloem degeneration, reduced root growth, reduced cane growth, delayed phenology, reduced yield, reduced fruit quality, delayed sugar accumulation, higher titratable acidity, lower anthocyanin content, lower pH, and lower wine quality (1). Reported findings on the impact of GLRaV-3 on white wine grapes are more limited. Little has been reported on the effect of GLRaV-3 on Marlborough-grown Sauvignon blanc, the flagship white wine of the New Zealand industry. We investigated the physiological responses of Sauvignon blanc vines to the presence of GLRaV-3 under field conditions.

MATERIALS AND METHODS

Sauvignon blanc vines grafted on SO4 rootstock with and without virus were monitored for three seasons. The vines were selected from an established vineyard on deep Rapaura series alluvial soil, planted in 1995 with mass selected clone material.

In 2006, 800 vines were enzyme-linked immunosorbent assay (ELISA) tested and healthy vines (no detectable GLRaV-3) were selected. Vines were also tested for GLRaV-1 and GVA and any vines with these viruses were excluded from the design. Selected healthy vines were tested three times (February 2006, July 2006, and June 2007). Three newly infected vines were detected in June 2007 in the previously GLRaV-3-free vines and these vines were added to the GLRaV-3 vine pool for crop analysis for 2007 and 2008.

Field assessments of leaf chlorophyll content were made on 15 March 2006 for each of the individual vines, using a Minolta SPAD-502 Chlorophyll Meter.

In all seasons, maturity (soluble solids content, measured as °Brix) and bunch parameters (average berry weight, bunch number, total yield) from all individual vines were assessed at harvest. Harvest dates were 20 March 2006, 30 March 2007 and 28 March 2008. For the maturity assessments the berry samples were crushed and the juice was used to determine the soluble solids concentration with a temperature-compensating refractometer (pocket pal-1, Atago). Bunch number and total yield per vine were recorded and average bunch weight calculated.

Analysis of variance (ANOVA) was performed for each attribute.

RESULTS

At harvests in 2006, 2007 and 2008, vines infected with GLRaV-3 had significantly heavier berries than fruit from vines without GLRaV-3. However, no significant differences in total yield or soluble solids contents were detected (Table 1). In 2007, bunch number per vine was significantly lower on vines infected with GLRaV-3.

In 2006, leaf chlorophyll content was significantly lower in leaves from vines infected with GLRaV-3 (Table 1).

Table 1. Mean physiological measurements at harvest for all sites and seasons of Sauvignon blanc grapevines, with and without detectable *Grapevine leafroll-associated virus 3*.

	2006		2007		2008	
	+	-	+	-	+	-
	n=20	n=20	n=43	n=37	n=43	n=37
SPAD ¹	23.1	28.3	nm	nm	nm	nm
Average	2.33	1.983	2.204	2.052	2.502	2.292
berry weight						
(g)						
Bunch	53.1	58	59.1	67.6	57.8	55.5
number per						
vine						
Total yield	5.6	6.5	5.7	6	9.2	8.8
per vine (kg)						
Soluble	21.7	22.1	20.5	20.8	21.4	21.6
solids (°Brix)						

¹SPAD is a relative measurement of leaf chlorophyll content; higher values indicate more chlorophyll. No measurements (nm) were made in 2007 or 2008.

+ With detectable **GLRaV-3**, - Without detectable **GLRaV-3**.

Means in **bold** were significantly different (*P* value <0.05) when compared within the season.

DISCUSSION

In this preliminary study, GLRaV-3 did affect the size of the Sauvignon blanc berries in all three seasons, but the results were not typical of the symptoms reported for red grapes. Further study is required to assess if the variety or climate were responsible for the lack of impact of GLRaV-3 on Sauvignon blanc grapes in Marlborough.

ACKNOWLEDGEMENTS

This project was made possible with the assistance of Willie Crosse who allowed access to his vineyard. Funding for the project was provided by New Zealand Winegrowers.

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Beet western yellows virus in canola crops: high infection in south-eastern Australia

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INTRODUCTION

A wide spread infection of *Beet western yellows virus* (BWYV) [(Syn. *Turnip yellows virus* (TuYV))] was detected in South Australia (SA), Victoria (VIC) and New South Wales (NSW) in 2014. BWYV is the most important virus disease of canola which can decrease yield by up to 46% (1). It is exclusively transmitted by aphids with the green peach aphid (GPA) (*Myzus persicae*) considered to be most efficient vector. The other viruses reported in canola in Australia are *Turnip mosaic virus* (TuMV) and *Cauliflower mosaic virus* (CaMV).

MATERIALS AND METHODS

During July-September, large surveys of over 600 canola crops were conducted and samples were either collected randomly or from symptomatic plants from commercial paddocks and field trials from SA (8088), NSW (4359) and Victoria (1338) and tested for BWYV, using tissue blot immunoassay (TBIA). All NSW samples were also tested for TuMV and 2000 samples tested for CaMV. Six hundred Victorian and SA samples were also tested for TuMV and CaMV.

RESULTS

Early in the season, BWYV incidence recorded in mid north of SA, ranged between 50-100%. The large surveys conducted later in the season in NSW, SA and Victoria indicated that the incidence of virus was 49, 84 and 50 percent respectively (Figure 1). BWYV distribution and testing results are shown in a map (Figure 2). TuMV was only detected in a limited number of locations in NSW (Liverpool Plains, Forbes, Coolah, Greenethorpe), confirming earlier reports that canola-virulent TuMV strains were not yet widely distributed in Australia (2). None of the tested samples reacted positive to CaMV. The Victorian and SA samples tested for TuMV and CaMV were negative. During the surveys in SA, 1043 weed and alternative host samples were also collected and tested of which 175 samples were positive to BWYV. The main weed hosts found were; wild turnip, turnip weed, Indian hedge mustard and marshmallow with virus incidence of 84%, 67%, 30% and 27% respectively.

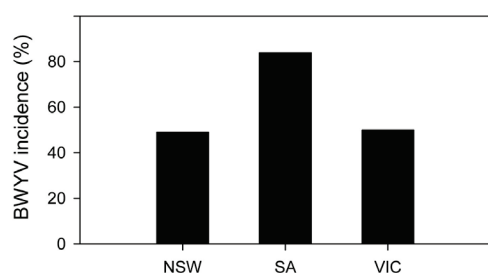


Figure 1. Incidence of BWYV in canola in south eastern Australia (NSW, SA and VIC) in 2014.

DISCUSSION

BWYV incidence was highest in SA, likely related to unusual high green peach aphid (GPA) activity during May and June (unpublished data). Possible trigger factors for the high aphid population as well as the resulting BWYV epidemic could be (i) summer rainfall resulting in a green bridge for virus and aphid vectors, (ii) mild autumn conditions viz. 2°C warmer than long term averages, (iii) large flights of green peach aphid in early season, (iv) limited use of insecticide seed dressings and (v) the prevalence of insecticide resistance in green peach aphid (3). At present virus resistant varieties of canola are not available so integrated management practices are advised: weed control, removal of green bridge during summer, sowing into standing stubbles, insecticide seed dressings and in crop aphid control based on monitoring.

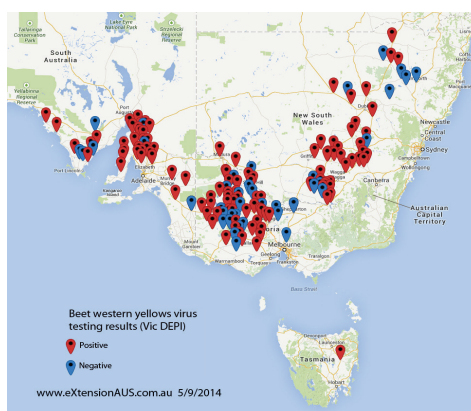


Figure 2. Distribution of BWYV in south eastern Australia from virus testing results.

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Measuring plant response to virus infection and water stress using quantitative composite indices

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We have developed an integrative plant-centric growth analysis approach to perform a 'meta-analysis' style comparison of plant responses to virus infection, across virus strains and host species. Our tool uses measures of plant development to calculate three normalised composite indices: for vegetative growth; reproductive development; and symptom intensity—be they disease-related or due to abiotic stress. These indices allow quantification of how host-virus interactions vary relative to each other, and how this then affects other plant responses within the context of an interactive disease model that recognises that host and virus are both active 'game' players. Such analysis offers a complementary perspective to molecular and serological information, which may not correlate directly with disease symptom intensity, or virus transmission and host performance.

The analysis approach has been applied to data series from *Arabidopsis thaliana* and *Nicotiana benthamiana*, infected with a range of viruses (*Cucumber mosaic virus*, *Potato virus A*, *Tobacco ringspot virus*, *Turnip mosaic virus*, Turnip vein clearing virus, Turnip yellow mosaic virus), observed up to 28 days post inoculation. It was also applied to data for +/- TRSV-infected *Nicotiana glutinosa* subjected to periods of water stress, to test whether infection provided a beneficial protective effect. Results show that vegetative growth, reproductive development, and symptom expression vary in their sensitivity to infection, depending on particular host-virus combinations, and that investigation of water stress protective effects from infection needs to consider indirect effects via reduction in plant growth rate.

POSTER 1

The genetic basis of sensitivity of barley to toxins produced by the net blotch pathogen *Pyrenophora teres f. teres*

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INTRODUCTION

Net form net blotch (NFNB) disease is capable of producing severe damage to the yield of barley crops. For more than 30 years, *Pyrenophora teres f. teres* (*Ptt*) has largely been controlled by breeding and cultural practices. However more recently, disease resistance available in a number of commonly grown cultivars has been overcome by the pathogen leading to higher disease levels. *Ptt* has been shown to produce fungal proteinaceous toxins which act in a host-specific manner (that is, the proteins only induce symptoms on barley cultivars and lines considered susceptible to net blotch disease) (1). These proteinaceous toxins (also known as effectors) probably target a host protein in susceptible barley plants conferring sensitivity. In resistant plants, the target protein may be absent or differ in a way that prevents alteration by the effector. Alternatively, the alteration of the target by effectors may trigger a defence response. An understanding of the plant response to toxins and the host target of the effectors may be important in breeding for disease resistance to NFNB. This research primarily aimed to determine whether toxins (and/or effectors) can be used directly as a selection tool for the development of NFNB-resistant germplasm. Furthermore, quantitative trait loci (QTL) for toxin sensitivity (and therefore potential host targets) have also been identified.

MATERIALS AND METHODS

Twenty-five single-spore *Ptt* isolates were chosen for proteinaceous toxin production based on a number of parameters: the variety from which the isolates were collected, when isolates were collected, and how virulent they were on key varieties and breeding lines. Isolates of *Ptt* were grown in Fries culture medium (FCM) and proteinaceous toxin extracts prepared using filtration (1). Individual recombinant proteins were also produced using an *E. coli* expression system (Invitrogen). Resistance ratings of 24 barley varieties were established by pathotyping of seedlings and adult plants with a number of isolates over a number of years and categorised as VS=very susceptible, S=susceptible, MS=moderately susceptible, MR=moderately resistant, or R=resistant. Sensitivity of barley varieties to the toxin extracts or recombinant proteins was determined by injecting 10 µg of protein into attached leaves using a Hagborg device and the extent of symptom development scored using a scale of 1 to 5. A consensus sensitivity rating was then determined for each variety using all 25 toxin extracts. Toxin sensitivity of a number of parents of mapping populations and key barley pathotyping differentials was also determined. Because the toxin sensitivity was differential between the varieties Mundah and Keel, a double haploid Mundah/Keel mapping population was screened with toxin extracts from the most virulent isolates to identify potential QTL. A set of Mundah/Keel recombinant inbred lines (RILs) with recombination in the QTL regions were also screened to further define QTL.

RESULTS AND DISCUSSION

The extent to which whole toxin extracts cause symptoms varies between varieties and the pathotypes from which toxins were extracted but not always in a correlated manner as expected. This probably reflects the impact that fungal behaviour has (that is, its ability to germinate, produce hyphae and spread throughout the plant tissue) (2) and which toxins are produced during the interaction and the timing of their production. In addition, although all isolates appear to be capable of producing a number of toxins in culture, the amount that they produce during the actual plant-pathogen interaction can vary depending on the pathotype. For example, even though a range of pathotypes can produce endoxylanase in culture, the most virulent isolates produce endoxylanase to a greater extent during the plant pathogen interaction (3).

The consensus toxin sensitivity rating was the same as the consensus resistance rating for 21 of the 24 varieties suggesting that whole toxin extracts from multiple isolates may be of use in screening germplasm for resistance to NFNB.

The ability to differentiate toxin sensitivity between parents of mapping populations has also allowed us to determine if regions of the genome contribute to this sensitivity. When screening with whole toxin extract from an isolate virulent on most varieties, significant QTL were detected on 1H (LRS = 31) and 7HS (LRS = 48). In both cases, the effect was in the direction of sensitivity from Mundah. The effects of these QTL seemed large enough to explain most of the difference observed between the parents. Anchorage of the QTL positions to the physical sequence assembly, will allow potential targets of the toxins to be identified for further research.

ACKNOWLEDGEMENTS

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POSTER 2

Full sequencing of an isolate of tomato yellow leaf curl virus from Iran and study of its phylogenetic relationship

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INTRODUCTION

Tomato yellow leaf curl virus (TYLCV) is one of the most severe diseases of cultivated tomato worldwide, a geminivirus transmitted by the whitefly *Bemisia tabaci*. Here we obtained genome sequences of an isolate from Iran.

MATERIALS AND METHODS

For full genome sequencing and determination of taxonomic position of tomato yellow leaf curl virus isolated from the Aabdan, Kangan and Asalooyeh of Boushehr province, 28 samples of tomato was collected from tomato farms of these regions in the winter of 1391. Using degenerate primers of begomoviruses (primer B^C/PCR^V 181) a fragment (500bp) was amplified in Asalooyeh sample in polymerase chain reaction (PCR). Using the sequence of this fragment, a primer pair was designed (Fars^C/Fars^V) to achieve the full genome sequence of the virus. Using the combination of primers (Fars^C/PAL1^V 1978) / (Fars^V/TYLCV-[Ab] 1997^C) complete sequence of the Asalooyeh virus was determined.

RESULTS

Using Bioinformatics programs and the comparison of complete genome of the virus with other viruses in Gene bank the taxonomic position of Asalooyeh isolate was determined. This is a member of Omani group of tomato yellow leaf curl virus.

DISCUSSION

According to the obtained dendrogram and proximity of this isolates with Arad isolate of Larestan shows the possible movement of virus between border regions of Fars and Boushehr provinces.

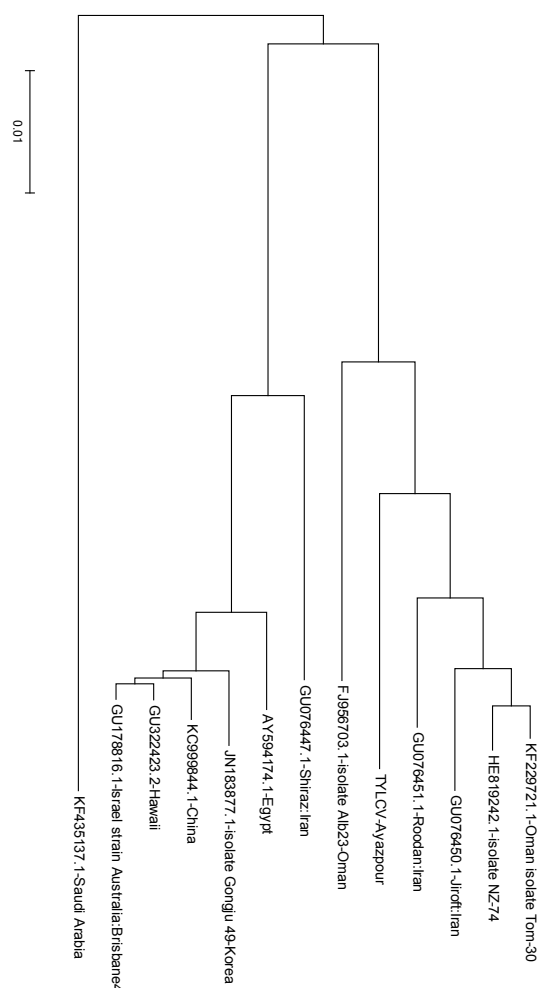


Figure 1. Phylogenetic dendrogram of Asalooyeh (TYLCV-Ayazpour) isolate comparing with some isolates around the world.

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POSTER 3

Temperature and moisture content stimulate the growth of fungi on healthy stored grain

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INTRODUCTION

Storage fungi can be present on grain at low levels at harvest, and also on grain handling and storage equipment and structures. If fungal growth proliferates it can increase the temperature of the grain, which in turn attracts insects and mites to the warm, humid conditions. In turn, insect respiration will produce further moisture to stimulate more fungal growth (1). Fungi can significantly reduce the quality of the grain and may also produce mycotoxins, rendering the grain unsuitable for human or animal consumption (2). The aim of this study was to investigate abiotic factors contributing to growth of fungi during grain storage.

MATERIALS AND METHODS

Grain was stored at three moisture contents (11, 13 or 15%) at three temperatures (20, 25 or 32.5°C) for 1, 2 or 6 months. There were 3 replicates for each time/temperature/moisture content combination and the experiment was repeated twice. Controls were grain sterilised by gamma-irradiation for one week at 30,000 GY. At each time period, 10 grains from each replicate were plated onto potato dextrose agar and the number of grains that exhibited fungal growth after 2 days at 25°C was recorded.

RESULTS

This study has demonstrated that fungi can be isolated from apparently healthy grain for up to 6 months when stored at higher moisture content and temperature. The ability to isolate fungi from stored grain decreased with increasing storage time.

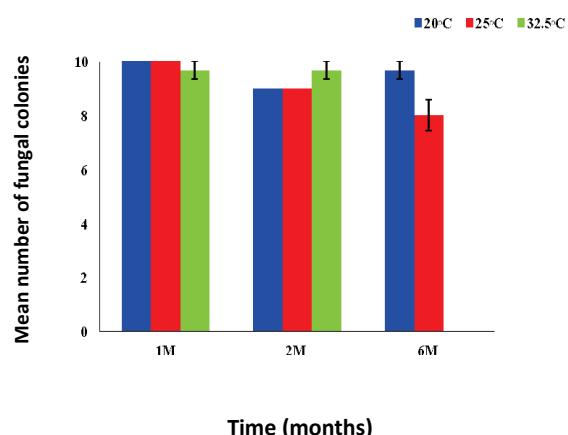


Figure 1. Mean number of fungal colonies isolated from wheat stored at 11% moisture content at 20°C, 25°C, 32.5°C for 1, 2 and 6 months. Bars represent standard errors of the mean.

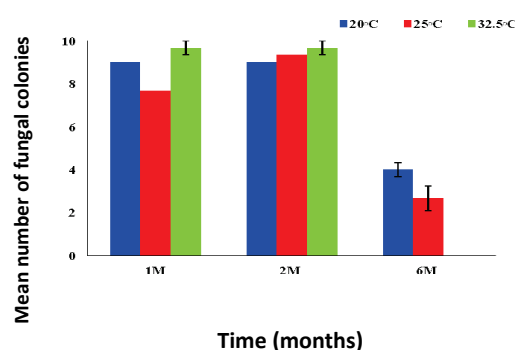


Figure 2. Mean number of fungal colonies isolated from wheat grain stored at 13% moisture content at 20°C, 25°C, 32.5°C for 1, 2 and 6 months. Bars represent standard errors of the mean.

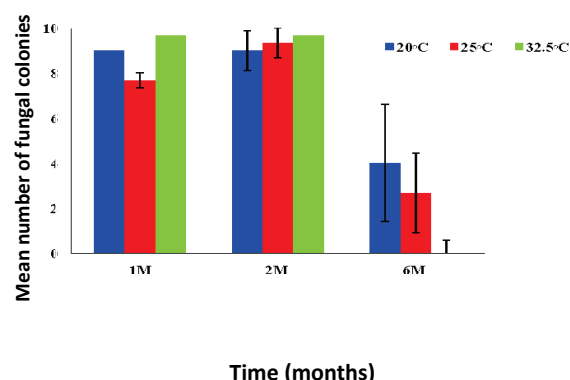


Figure 3. Mean number of fungal colonies isolated from wheat grain stored at 15% moisture content at 20°C, 25°C, 32.5°C for 1, 2 and 6 months. Bars represent standard errors of the mean.

DISCUSSION

Postharvest losses of grain caused by mould activity depends on several factors such as grain moisture content, storage temperature, the storage time, and the degree of contamination. We hypothesise that the fungi begin to lose viability after 6 months in storage if correctly stored.

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The first author would like to thank Murdoch University and the Libyan government for financial support.

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POSTER 4

The detection of postharvest fungi on wheat grain using volatiles

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INTRODUCTION

Volatile organic compounds (VOCs) can be released from commodities after harvest and during storage, directly from the commodity itself or as a result of insect pest and microbial infestations (1). The contents of volatiles are related to the history of the grain (pre-harvest conditions and treatments during storage). Therefore, the volatile compounds produced by fungi might be used to distinguish fungal growth in the grain. The aim of this study was to evaluate the detection of fungi in stored wheat from different regions in Australia by analysing for the production of specific VOCs related to different fungal species.

MATERIALS AND METHODS

Two *Alternaria* species regularly isolated from grain were examined in detail to determine if they produced specific VOCs in stored grain. These were added to sterile wheat at 15% moisture content incubated at 25°C for 10 days. The VOCs analysis was performed on a Hewlett Packard 6890 series gas chromatograph coupled to a flame ionisation detector (FID). The fibre was exposed to the headspace of the flasks at 45°C for three hours. GC-MS analysis was conducted using an Agilent 6890 gas chromatograph equipped with the 30 m × 0.25 mm × 0.25 µm Stabilwax® polar column that was coupled to an Agilent 5973 Network mass selective detector (MSD) with an Agilent ChemStation.

RESULTS

We found: (a) unique volatile metabolites specific to wheat; (b) metabolites common only to spoilt grain; and (c) high abundance of metabolites in contaminated grain compared to non-contaminated. For each *Alternaria* species, specific VOCs were produced (Fig 1, 2).

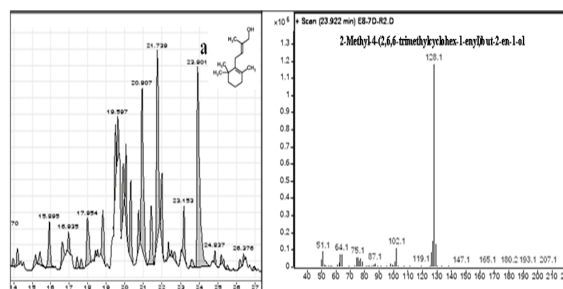


Figure 1. *Alternaria cf. infectoria* isolate A. GC-MS chromatogram (on the left) and positive-ion-mode scan spectra of the specific VOCs (on the right).

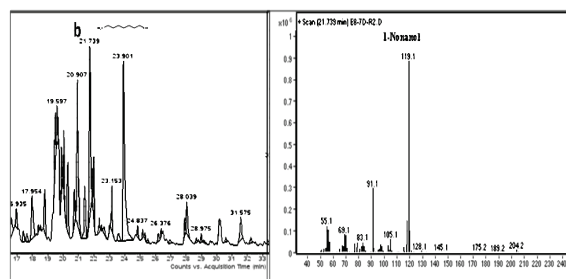


Figure 2. *Alternaria cf. infectoria* isolate B. GC-MS chromatogram (on the left) and positive-ion-mode scan spectra of the specific VOCs (on the right).

DISCUSSION

The present study considered specific criteria based on volatile metabolite profiles to discover and discriminate among pathogenic fungi on wheat.

This study shows that VOCs can be used to identify fungi to a special level. This method is rapid, accurate and cost effective.

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POSTER 5

Viability of plant pathogen fungal spores over time at different temperatures carried by tested materials and their relative abilities and effectiveness as spore carriers

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INTRODUCTION

Fungal pathogens are a major cause of yield losses across agricultural crops and fungal spores plays a major role in the disease development and spread of a disease (1, 2).

While fungal spores may be primarily dispersed by wind or rain, dispersal can also occur through human activities allowing pathogens to spread to new geographic areas (3,4). To understand and study the different entry pathways of fungal spores, it is critical to investigate the possibility of how different materials act as spore carriers. In general, data regarding the role of different materials as a fungal spore carrier remains very limited. The current study demonstrates the abilities and effectiveness of different materials as fungal spore carriers and spore viability on tested materials.

MATERIALS AND METHODS

Pathogen information and inoculation. Twenty different materials were selected to be inoculated with the selected 'model' pathogens: *Puccinia striiformis* var. *tritici*; *Leptosphaeria maculans*; and *Kabatiella caulivora*. Under selected environmental conditions, spore numbers and survival of spores from these materials as spore carriers were measured. Test materials were inoculated with a concentration of 10^4 spores/ml of each pathogen separately and placed in three controlled environmental rooms as follows: 24°C day/8°C night, 36°C day/14°C night and 45°C day/15°C night. The experiments are designed to run for 12 months in order to observe the effects of temperature on spore survival over time course. Periodic sampling started from 24 hrs to 8 months to determine the effectiveness of different materials as spore carriers.

Isolation of spores from the inoculated materials. Treated spores were isolated from the tested materials (after inoculation) washing with 0.1% Tween 20 and the residue used for determination of total number of spores obtained and for observation of the cellular activity of live spores. Non-inoculated materials were served as control.

Spore counting and viability test. Numbers of spores were counted in residual solutions after washing to determine the efficiency of each of the materials as a spore carrier. Total number of spores present and numbers of spores remaining alive were determined. Samples collected from residual solutions were used to monitor cellular activity of live spores.

RESULTS AND DISCUSSION

Results demonstrate that fungal spores can remain attached to different materials and viable for at least 8 months. There was a clear interaction between the type of the materials and the spore size and morphology. Temperature and light conditions over time impacted on the viability of spores. Microscopic studies confirmed that some spores can germinate rapidly and form resting

hyphae. Cellular activity was observed in spores which did not germinate even after over 8 months.

To understand the role of different materials is critical in the entry and dispersal of fungal pathogen. That determines the success of pathogen introduction and also helps developing mitigation programmes and decontamination processes for biosecurity.

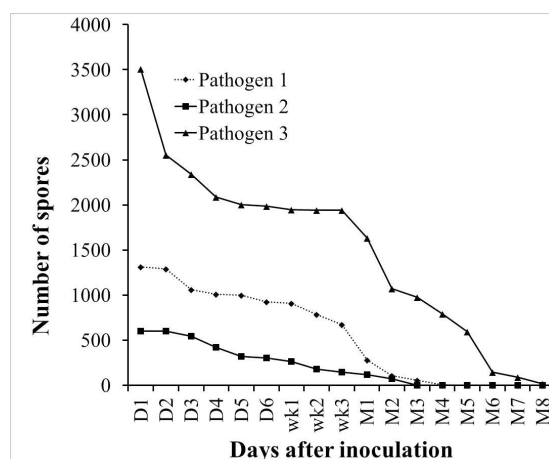


Figure 1. Viability of spores over time period of eight months on steel at temperature 23°C day/8°C night (Pathogen 1- *Leptosphaeria maculans*; Pathogen 2 - *Kabatiella caulivora*; Pathogen 3 - *Puccinia striiformis* var. *tritici*)

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POSTER 6

Host preferences of *Pratylenchus quasitereoides*—a new challenge in soil-root battlefieldFarhana Begum^{A,B}, Shashi B. Sharma^A, Michael Jones^{A,B}, and Bill Macleod^C^ASchool of Veterinary and Life Sciences and ^BWA State Agricultural Biotechnology Centre, Murdoch University, Perth WA 6150, Australia, ^CDepartment of Agriculture and Food Western Australia, South Perth, WA 6151, Australia

INTRODUCTION

Pratylenchus quasitereoides is a newly identified root lesion nematode (RLN) species in Australia (Hodda *et al.* 2014). This species is present in Western Australia from Carnamah (29°41'S 115°53'E) to Tambellup (34°02'S 117°38'E) (Collins *et al.* 2013; Vanstone 2007; Riley & Kelly 2002), and in South Australia (J Nobbs, SARDI, personal communication). Preliminary studies have shown that *P. quasitereoides* is a highly damaging nematode species and it is capable of causing much greater yield losses in wheat and barley than other common species of RLN (Carla *et al.* 2014).



The host preferences of this new species have not yet been studied, therefore, the present study was carried out to evaluate selected crop species and cultivars for their ability to host *P. quasitereoides*.

MATERIALS AND METHODS

A total of 19 different crop and pasture species, belonging to 5 different families were evaluated under controlled environment conditions in a glasshouse for their ability to host *P. quasitereoides*. The nematode population initially collected from the Department of Agriculture and Food Western Australia, was further multiplied on barley cultivar Stirling at Murdoch University. Seeds of test genotypes and control susceptible host (barley cv Stirling) were sown in plastic pots containing 1000g autoclaved soil. Seedlings of 8 days old were inoculated with 1000 nematodes per pot. After eight weeks of nematode inoculation, soil and roots from three plants were processed from each pot to extract nematodes using misting technique. Nematode data were recorded and analysed using MSTAT and different genotypes were compared following Duncan's New Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

P. quasitereoides population was found in the roots of all the tested plant species (Fig. 1). The average nematode number was lowest on forage brassica, sub clover and rye grass. When compared with barley cv Stirling, two genotypes-Barley cv Hamelin (Fig.2) and Sorghum cv Superdan II were identified as good host in terms of number of nematode per gram dry root. Though produced less number of *P. quasitereoides* compared to control, the rest of genotypes found as statistically similar. Number of *P. quasitereoides* varied greatly between hosts, and even within cultivars of the same species. This research has identified barley, sorghum, millet, lupin, chickpea, oat, canola and wheat as hosts of this species. This new knowledge of host preferences will enable growers and consultants to make appropriate cropping choices in *P. quasitereoides* infested soils.

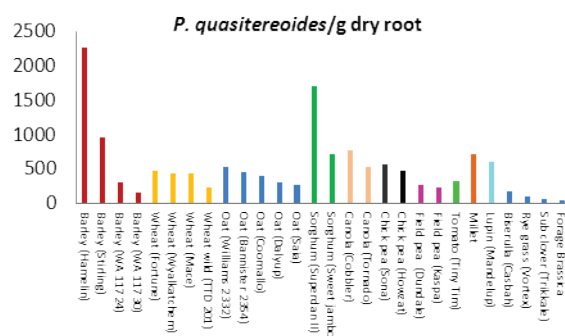


Figure 1. Hosting ability of different crops to *P. quasitereoides*



Figure 2. Infected barley roots (left) and stained roots with nematode inside (right)

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POSTER 7

Effect of break crops on root lesion nematode levels

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INTRODUCTION

The root lesion nematode (RLN) species *Pratylenchus thornei* and *P. neglectus* are significant soilborne pathogens of broadacre crops in southern Australia.

Management of RLN is based on the use of resistant varieties and crops. Most of the research undertaken so far has focussed on exploiting genetic variation in wheat and barley. RLNs have a broad host range so research is now being expanded to include crops grown in sequence with wheat and barley.

This paper summarises results from two years of monitoring *P. thornei* and/or *P. neglectus* multiplication in selected national variety trials (NVT) in SA targeting crops grown in sequence with wheat and barley.

MATERIALS AND METHODS

Each NVT site in SA and Vic was sampled at or prior to seeding and tested by PreDicta B to identify trials with low to medium levels of *P. thornei* and/or *P. neglectus*.

Initial and final RLN population densities were assessed in each plot by collecting separate soil samples from the selected trials soon after seeding and again post-harvest. Each sample was a composite of 21 soil cores 0-10 cm; 3 cores were taken from 7 locations equidistant along the plot. Post-sowing cores were collected between the rows and post-harvest cores on the rows. Soil samples were processed by the SARDI Molecular Diagnostic Centre

Data were analysed using linear mixed models and residual maximum likelihood estimation to produce best linear unbiased predictors for each variety. The analysis checks and corrects for spatial variability to calculate predicted multiplication rates and assesses for genetic variation across the varieties.

RESULTS AND DISCUSSION

Canola was assessed at one site only but all entries were susceptible to *P. neglectus*, and there were no significant varietal differences.

Desi and kabuli chickpeas were assessed at Riverton 2014; both crops were less susceptible than expected to

P. neglectus and *P. thornei*, possibly due to the lack of rain in late winter and spring.

Faba beans were susceptible to *P. thornei* in 2013, but behaved as a resistant crop in 2014, however there were significant variety effects in 2014 with multiplication rates ranging from 0.4 to 0.8. All entries were resistant to *P. neglectus* in both years.

Field peas reduced levels of *P. neglectus* and *P. thornei* in 3 and 2 trials, respectively. There were also significant varietal differences for *P. thornei* multiplication at Riverton ranging from 0.5 to 0.7.

A summary of RLN multiplication in the canola, chickpeas, faba beans, field peas, lentils, oats and triticale trials assessed in SA is presented in Table 1. Of the 14 trials sampled, 9 had mixed populations of *P. neglectus* and *P. thornei*.

Lentils appear to be moderately susceptible to *P. neglectus* and *P. thornei* with mean crop multiplication rates reaching around 2 at the higher end.

Oats increased *P. neglectus* levels 4-fold in the 2014 trial at Paskeville.

Triticale reduced *P. neglectus* levels at the Pinnaroo trial and there were significant varietal differences in multiplication rates ranging from 0.2 to 0.9.

Data obtained show significant crop differences in susceptibility to *P. neglectus* and *P. thornei* and that the genetic x environmental effect on RLN multiplication maybe greater for pulses than with cereals. Environmental conditions may also affect carryover of RLN between seasons. As more trials are assessed, MET analyses will be used to assist in classifying RLN resistance for crops/varieties grown in sequence with wheat and barley. This information will assist growers to plan more effective management programs.

ACKNOWLEDGEMENTS

Grains Research and Development Corporation (GRDC) for funding the research. Project code: DAV00128.

Table 1. Mean crop effects on RLN multiplication in South Australian NVT sites

Crop	Year	Location	Pi	<i>P. neglectus</i>		<i>P. thornei</i>	
				Multiplication	Pi	Multiplication	
canola	2013	Artherton	8.3	8.2			
chickpea (desi)	2014	Riverton	1.6	0.3	16.4	0.8**	
chickpea (kabuli)	2014	Riverton	2.4	0.6	17.3	1.1*	
faba Bean	2013	Maitland	4.7	0.4	3.8	9.2	
faba Bean	2014	Laura	8.8	0.7	5.2	0.6*	
field Pea	2013	Willamulka	10.5	0.1			
field Pea	2014	Laura	7.1	0.4	1	0.3	
field Pea	2014	Riverton	3.3	0.3	13.6	0.6*	
lentil	2013	Maitland	5.9	2.2	2	2.1	
lentil	2013	Willamulka	13.6	1.1			
lentil	2014	Laura	7.4	0.9	7	0.5	
lentil	2014	Riverton	5.2	0.7	21.2	0.7	
oat	2014	Paskeville	1.5	4.0			
triticale	2014	Pinnaroo	7.3	0.4**			

Significant differences in RLN multiplication rates between entries is indicated by * (P < 0.05) and ** (P < 0.001)

Pi = initial nematode population at seeding (/g soil)

POSTER 8

Population structure and infection biology of *Phytophthora pluvialis* in *Pinus radiata* forests in New Zealand

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INTRODUCTION

Phytophthora is an important genus of pathogens that are responsible for some of the most damaging plant diseases worldwide. *Phytophthora pluvialis* is a foliar pathogen that was recently identified as the causal agent of red needle cast on *Pinus radiata* in New Zealand, the symptoms of which are shown in Figure 1. *P. pluvialis* was first discovered in New Zealand in 2008 but had previously been recovered from Tanoak and Douglas fir trees in Oregon (USA) in 2002. *P. pluvialis* was subsequently described as a new species in 2013 (Reeser et al., 2013) and is classified in *Phytophthora* clade 3 based on the ITS region. In Oregon *P. pluvialis* has been isolated from soil, streams and canopy drip, and seven unique haplotypes were identified using the *cox* spacer sequence (Reeser et al., 2013).

The purpose of this project is to determine the population structure and mechanisms that make *Phytophthora pluvialis* a successful foliar pathogen of *P. radiata* in New Zealand. This will be achieved through three objectives; the first objective is to assess the genetic diversity of *P. pluvialis* in New Zealand. The second objective is to look at gene expression of *P. pluvialis* at different time points after infection. The final objective is to use microscopy techniques to observe the lifestyle and infection process of *P. pluvialis*.

MATERIALS AND METHODS

A collection of 148 *P. pluvialis* isolates from Scion (Rotorua, New Zealand) are being used for this study. The isolates were verified as *P. pluvialis* by using species specific primers Ypap2F and Ypap2R (R. L. McDougal, unpublished), targeting the *ypt1* gene.

For a preliminary analysis of genetic diversity, the *cox* spacer region was amplified using *Phytophthora* specific primers FMPh8 and FMPh10 (Reeser et al., 2013).

The PCR products were sequenced by Macrogen Inc (Seoul, Korea <http://dna.macrogen.com/eng/index.jsp>) in both the forward and reverse direction using primers FMPh8 and FMPh10. The *cox* spacer sequences from the New Zealand population were aligned to the seven Oregon haplotype sequences using Geneious version 8.0.3 (<http://www.geneious.com>, Kears et al., 2012) and assessed for similarities.

RESULTS AND DISCUSSION

Screening with the Ypap primers confirmed the identity of 146 *P. pluvialis* isolates. Comparing 313 bp of sequence from the isolates, it was observed that 128 individuals had the same *cox* spacer sequence indicating that the *P. pluvialis* population in New Zealand is very homogeneous. The remaining isolates need further sequence confirmation. When comparing these 128 sequences to the seven haplotypes found in Oregon all the New Zealand samples had the same *cox* spacer region sequence as Oregon sample MDR-5-010307 (haplotype 3) which was isolated from a raintrap in 2007 (Reeser et al., 2013).

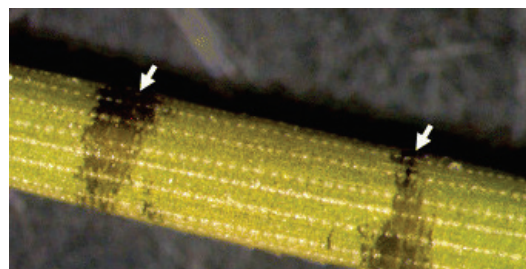


Figure 1. *Pinus radiata* needle showing olive green banding which is characteristic of red needle cast symptoms caused by *Phytophthora pluvialis*. (Dick et al 2014)

FUTURE WORK

Although the *cox* spacer region of *P. pluvialis* from New Zealand showed no variation, a broader study will be done to identify single nucleotide polymorphism (SNP) markers at other loci with which to assess the genetic diversity of *P. pluvialis* in New Zealand. The markers will be identified based on heterozygosity from two available *P. pluvialis* genomes: one a *P. pluvialis* isolate from New Zealand and the other from Oregon. For this study, the target is to identify 30 SNP markers across the *P. pluvialis* genome. The SNPs will be screened on a panel of eight samples; six from New Zealand and two from Oregon. Only the SNPs that are heterozygous in New Zealand will be used in this study.

ACKNOWLEDGEMENTS

I would like to thank Wendy Sutton (Oregon State University) and David Studholme (University of Exeter).

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POSTER 9

Getting to the root of the problem: *Rhizoctonia solani* in Champasak, Lao PDR

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INTRODUCTION

Vegetable production in the Lao PDR has been gradually transitioning from production on very small farms or gardens for the local village or town markets to larger small-holder farms (one to five ha) or polyhouse operations since about 2008. This trend is particularly evident in Champasak province where vegetable production is now concentrated on the Bolavan Plateau at 1000 to 1200m altitude. The altitude moderates the climate compared with the typical wet dry tropical climate of the lowlands along the Mekong River. The plateau has fertile volcanic soils and areas of remnant rainforest. A wide range of vegetables is being produced on the plateau with small areas in the lowlands. Round-head cabbage is the main field crop being produced by small-holders on the plateau for consumption in the Lao PDR and export to Thailand. In contrast other vegetables such as tomatoes, chilli, lettuce, cucumbers, and eggplants are mainly being produced in small polyhouse operations as well as in several large operations.

Collar rot was first observed in the field on the plateau in cabbage seedling in 2009 and *Rhizoctonia solani* was isolated. As cabbages were the main field crop and collar rot was becoming more serious by 2012 we initiated a study to monitor the incidence and severity of the disease on a qualitative basis in small-holder cabbage fields, and to clarify the factors favouring the disease. Diagnostic studies were undertaken in parallel to confirm the presence of the pathogen in other crops and isolate cultures for deposition and sequencing to determine the anastomosis group, as described in (1).

MATERIALS AND METHODS

We conducted ad hoc surveys of cabbage fields each year in both the wet and dry seasons from 2012. Five roadside fields that could be surveyed each season have been monitored regularly. Some fields could not be accessed in the wet season. In each field at least one member of our team walked a minimum of 100m in a zig-zag pattern through the crop. Stunted seedlings or mature plants were selected on an ad hoc basis for removal to confirm the presence of collar rot. We also held discussions with the farmers where possible.

Ad hoc surveys were also undertaken in polyhouse farms of the common Brassica crops as well as other crops for the presence of the disease. Cultures of putative *R. solani* were recovered from all crop species affected by collar rot either at the seedling or older plant stages. Representative cultures were retained for deposition in the International Collection of Microorganisms from Plants (ICMP), Landcare Research, Auckland NZ, and sequencing to confirm the morphological identification and determine the anastomosis group (1). Pathogenicity tests are also being undertaken.

RESULTS AND DISCUSSION

All fields were in a cabbage monoculture as farmers had no profitable alternative crop. Field observations revealed a gradual decline in yield (head size) from 2009 to the present. Indeed by 2014 many fields had gone out of production as the small-holder owners sought the use of alternative fields with no history of cabbage production, or of fields that had been newly cleared of forest. The change in head size in one field between an initial crop in 2009 and a crop in 2014 will be illustrated in the poster. Indeed the senior author and the fourth author had not previously seen such severe stunting from collar rot in older cabbage plants in Australia. It was also observed that seedling production in all farms was in ground beds often in the field where seedlings were to be transplanted. Seedling losses from collar rot (wirestem) were significant. The fungus remained active in infected seedlings following transplanting. Fungicides were not being used as they are not readily available and their use is discouraged. In summary we believe that monoculture, the use of infected seedlings, the lack of fungicide use and poor sanitation have led to a gradual build-up in inoculum of *R. solani* in soil to high levels. *R. solani* was isolated from cabbages, gai lan, choy sum, bok choy, mustard greens, and pak choy and other crop species. An isolate from gai lan was determined to be anastomosis group AG-4 HG-1 (1). Work is continuing to clarify the anastomosis group(s) present in the province.

We have conducted farmer training in integrated disease management (IDM) and this is continuing with an emphasis on:

- crop rotation
- disease-free seedlings
- sanitation.

We are also working with stakeholders to encourage the import of appropriate fungicides for control of collar rot.

ACKNOWLEDGEMENTS

The support of the Crawford Fund of Australia, and the Office of International Programs of the American Phytopathological Society is gratefully acknowledged.

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POSTER 10

Down-regulation of the *Glucan synthase-like 6* gene (*HvGsl6*) in barley leads to decreased callose accumulation and increased susceptibility against *Blumeria graminis* f. sp. *hordei*

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INTRODUCTION

The recent characterisation of the polysaccharide composition of papillae deposited at the barley cell wall during an infection by the powdery mildew pathogen, *Blumeria graminis* f. sp. *hordei* (*Bgh*), shows that cellulose, arabinoxylan and callose are associated with penetration resistance of the wild type barley (1). The study suggests that callose could be one of the three potential polysaccharides that could be utilised for the generation of enhanced disease resistance.

The exact role of the callose found in the papillae of crop species is largely unknown as the genes involved in the observed callose accumulation have not been identified. In a number of crop species the *glucan synthase-like* (*Gsl*) gene family members have been reported to be involved in callose biosynthesis during specific growth and developmental stages. In the model species *Arabidopsis*, *AtGsl5* has been reported to be involved in accumulation of papillary callose during pathogen infection. It is likely that a functional ortholog of *AtGsl5* is present in crop plant species including barley.

In searching for the putative ortholog(s) of *AtGsl5* in barley we have utilised a comparative genomics approach that includes phylogenetic analysis, transcript profiling of the putative *HvGsl* family during pathogen infection, and functional analysis using loss-of-function strategies in transient and stable transgenic plants.

RESULTS

A phylogenetic analysis revealed that *HvGsl6* and *HvGsl7* have the closest homology with *AtGsl5*. Therefore, it is possible that either of these two genes are the potential orthologs of *AtGsl5*. Q-PCR expression profiling of *HvGsl6* and *HvGsl7*, conducted at different time points of infection by *Bgh*, revealed that only the *HvGsl6* transcripts significantly up-regulated during this time. Conversely, transcripts of *HvGsl7* were significantly down-regulated. Through a transiently induced gene silencing system (TIGS) we observed that papillae formed in *HvGsl6*_dsRNAi transformed cells have significantly less callose compared to wild type while *HvGsl7*_dsRNAi transformed cells showed no difference with wild type. These results suggest that *HvGsl6* is a potential functional ortholog of *AtGsl5*. For further confirmation *HvGsl6*_dsRNAi stable transgenic lines were developed, which show significantly lower level of *HvGsl6* transcripts. The reduced fluorescence intensity in the papillae when using a callose specific probe (aniline blue fluorochrome) suggests that the papillae formed in the *HvGsl6*_dsRNAi transgenic lines have significantly less callose compared empty vector and wild type lines (Figure 1B-D). The accumulation of callose near sites of mechanical wounding of the leaf was also significantly reduced in *HvGsl6*_dsRNAi transgenic lines (Figure 1A). The decreased callose accumulation in the *HvGsl6* down-regulated lines observed in wounded tissue and papillae is associated with

significantly higher susceptibility to penetration by *Bgh* compared to controls lines.

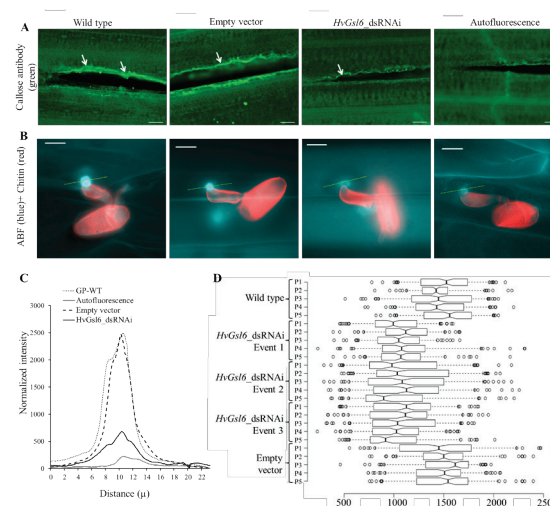


Figure 1. Down-regulation of barley *glucan synthase-like 6* in stable transgenic plants leads to lower accumulation of callose in wounded tissue and papillae formed in response to *Bgh* infection. A) Callose accumulation at artificially wounded sites 24 hai (green). B) Callose accumulation in the papillae formed near the barley-*Bgh* interaction sites at 3 dai. Callose was detected using the aniline blue fluorochrome (ABF, blue) and fungal structures were stained with Wheat germ agglutinin (red). Bars= 10 μ m. C) Fluorescence intensity profiles corresponding to readings across the yellow dashed lines in Figure 1B. D) Box plot showing normalised fluorescence intensity of aniline blue stained callose in barley papillae during *Bgh* infection.

DISCUSSION

The results show that the *HvGsl6* is a biotic and abiotic stress responsive gene in barley *Gsl* family, and is associated with both papillary callose and wound plug callose accumulation in the barley leaf. Down-regulation of *HvGsl6* leads to increased susceptibility against the biotrophic pathogen *Bgh*. This suggests that papillary callose is positively associated with barley penetration resistance. It is predicted that up-regulation of *HvGsl6* transcripts will lead to higher accumulation of callose in barley papillae and increased penetration resistance against *Bgh*. The association of polysaccharide deposition in the papillae and penetration resistance provides new targets for the generation of novel crop lines with greater disease resistance.

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POSTER 11

Extension of host range for two nematodes species in Australia

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INTRODUCTION

Plant-parasitic nematodes are a major constraint to agricultural and horticultural production worldwide. Worldwide crop losses due to nematodes on 37 crops are estimated at \$358.24 billion annually based on the 2010-2013 production figures and prices (1).

The detection, description, and recognition of possible new species and new hosts are highly relevant to practical nematologists and quarantine departments around the world as the spread of economically important plant-parasitic nematodes is still occurring today (2).

The nematode species *Hemicriconemoides mangiferae* (Siddiqi) has been recorded on a new host in Australia – mango, *Mangifera indica* L. The detection occurred on mango trees at Mutchilba, Queensland. *H. mangiferae* is highly polyphagous and is recorded on over 30 hosts worldwide. In Australia it is geographically widespread; in Queensland, it has been recorded on at least 17 hosts, one detection is recorded for New South Wales and three in the Northern Territory. In mango, the nematode feeds on the roots of affected trees as both an ecto and endoparasite (3). This nematode species is of economic importance to the mango industry in the USA, India, Pakistan and South Africa where it is associated with decline in mango. Symptoms of decline include reduced numbers of secondary roots, chlorosis and necrosis of leaves, leaf drop from branch tips, bare terminal twigs, dead branches, and occasional tree death (4).

The nematode species *Meloidogyne incognita* (Kofold & White, 1919) Chitwood, 1949 has also been recorded on a new host in Australia – cotton, *Gossypium hirsutum* L. The detection occurred in a cotton crop from the Ayr area in Queensland. There are more than 70 described species of *Meloidogyne*, but only *M. incognita* and one other species are known to be pathogenic to cotton. *M. incognita* is one of the species (2) and is common in tropical regions of the world with over 700 host listed (5). In Australia, it is known to occur on numerous hosts in every state. Of all the root-knot species, *M. incognita* has the greatest frequency distribution in warm temperate to tropical agriculture (2) and is of major economic significance throughout the warmer regions of the world. The general symptoms of disease include root galling while above ground symptoms include stunting, chlorosis, incipient wilting and a general unthrifty appearance.

MATERIALS AND METHODS

Hemicriconemoides mangiferae. Nematodes were extracted from soil sampled during a survey of a long term mango trial block in north Queensland. Two hundred grams of soil for each sample was placed in a Whitehead tray for 3 days for the extraction of nematodes (6). The resulting solutions were sieved using a 38 µm sieve and examined under a compound microscope for the identification and quantification of any plant-parasitic nematodes.

Meloidogyne incognita. Roots were sampled from an unthrifty area in a commercial cotton property in north Queensland. Egg masses picked from the galled roots were hatched in 5 mLs of water in a 26°C incubator over 5 days. After examination using a compound microscope, a tentative identification of *Meloidogyne* sp. was made of the hatched vermiform nematodes. For positive identification to species level, adult females were picked from the galled cotton roots and tested using PCR based DNA analysis (7).

RESULTS AND DISCUSSION

Hemicriconemoides mangiferae was identified in 7 soil samples from the mango trial block with very high numbers extracted ranging from 54 – 1,712/200 g soil. Studies of nematodes and mango decline have found that damage can be correlated to the density of *H. mangiferae* and that nematode populations are higher in lighter soils with excessive soil moisture (4). Further investigations are required to determine the extent of the nematode distribution and the level of damage the nematode is causing to mango production in Australia.

Meloidogyne incognita was identified from cotton roots grown in light sandy soils around Ayr. This species, known as the southern root-knot nematode, is the most damaging pathogen of cotton in the United States (8). RKN prefers sandy soils while much of the cotton in Australia is grown on heavy soils. This may limit the spread of this pathogen in Australian cotton.

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POSTER 12

Does soil acidity impact Western Australia's broadacre crops ability to resist root lesion nematodes?

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INTRODUCTION

Acid soils are prevalent across Western Australia's broadacre cropping areas and are known to impact on nutrient uptake and increase crop vulnerability to pests and pathogens. As more than 65% of broadacre cropping paddocks are infested with root lesion nematodes (RLN), a pest of cereals, pulses and oilseeds, the effects of pH on RLN multiplication was assessed. Glasshouse trials investigated whether soil acidity negated crop and cultivar resistance and increased the ability of *Pratylenchus neglectus* and *P. quasitereoides*, the most common RLN species in WA broadacre cropping, to multiply on commonly grown crops.

MATERIALS AND METHODS

Exp. 1 investigated the potential impact of low pH soil (acid soil) on resistance of wheat, barley, canola and lupin crops to *P. neglectus* or *P. quasitereoides*. For each crop, varieties with a range of RLN resistance were used. Soil came from a pH experimental trial paddock in Wongan Hills, where 'limed' and 'un-limed' strip plots are maintained (pH (CaCl₂) 6.7 & 5.1 respectively). Pots were inoculated (1000 RLN/pot) a week after germination and grown for 10 weeks in the differing pH soils in a glasshouse. Nematodes were extracted from the entire roots and counted microscopically.

Exp. 2 investigated the impact of acid soils on the resistance of wheat cultivars to *P. neglectus*. Soil from two field sites, with adequate (pH (CaCl₂) ~ 6.4) and low pH ((CaCl₂) ~ 4.7) strip plots were utilised. Wheat cultivars considered more resistant (Wyalkatchem, Yenda and Tamaroi) and susceptible (Calingiri, Wylie and Brookton) to *P. neglectus* were assessed using similar methods to **Experiment 1**.

RESULTS AND DISCUSSION

Final *P. quasitereoides* populations were significantly higher in barley, lupin and wheat crops grown in acid soil and in barley for *P. neglectus* (Fig 1) compared with RLN populations extracted from the same crops grown in the same soil type maintained at moderate pH.

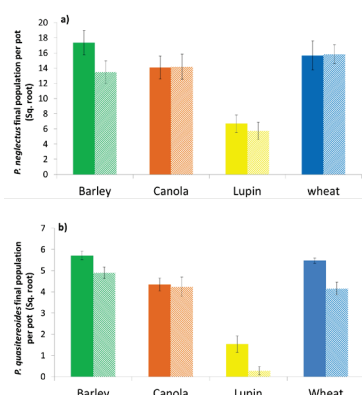


Figure 1. Impact of low (5.1, solid bars) and moderate (6.7, striped bars) pH soil on a) *P. neglectus* and b) *P. quasitereoides* final populations in wheat, barley, canola and lupin crops (n=6).

Soil acidity impacted variety resistance to *P. quasitereoides*. Significantly ($p < 0.05$) higher final populations were recorded for seven of the nine wheat, barley and lupin varieties grown in low pH soil compared to the same varieties grown in the same soil type with moderate pH.

For canola there was no effect of pH on multiplication of *P. quasitereoides* or *P. neglectus*. It is thought that there is a direct relationship between soil pH and plant stress which leads to an increase in multiplication of RLN. Canola is known to be tolerant of soils at the pH tested (5.1) so may not have been stressed and thus there was no increase in RLN multiplication.

The relationship between acid soils, and variety resistance to *P. neglectus* was not conclusive. In Experiment 1 there was only a significant ($p < 0.05$) increase in final numbers of RLN in two of the nine varieties tested in low pH soil compared those grown in the same soil type maintained at moderate pH. In Experiment 2, *P. neglectus* multiplication was higher in acid soil compared to moderate pH soil in six of the seven varieties tested regardless of inherent variety resistance (Fig 2).

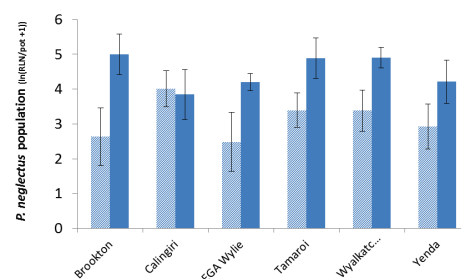


Figure 2. Impact of low (4.7, solid bars) and moderate (6.4, striped bars) pH soil on *P. neglectus* final populations in six wheat varieties with a range of *P. neglectus* resistance classifications (n=12).

Our experiments established that some varieties of WA's commonly grown crops; wheat, barley & lupin are more susceptible to *P. neglectus* and *P. quasitereoides* in low pH soil regardless of their usual resistance characteristics. This work builds on previous DAFWA research into impacts of WA's acid soils. The increasing practice of liming western Australian broadacre cropping soils may also decrease the detrimental effect of RLN on crop growth and yield.

ACKNOWLEDGEMENTS

We thank our DAFWA colleagues for help and expertise in soil science. This research is jointly funded by DAFWA and GRDC.

POSTER 13

Can spectral measurements be used to detect *Phytophthora*-related stress in *Corymbia calophylla* (marri)?L. Croeser^A, T. Paap^A, T. Burgess^A, G. Hardy^A and M. Andrew^B^A Centre for Phytophthora Science and Management, School of Veterinary and Life Sciences, Murdoch University, Perth, 6150, WA, Australia^B School of Veterinary and Life Sciences, Murdoch University, Perth, 6150, WA, Australia

INTRODUCTION

Widespread mortality of *Corymbia calophylla* (marri) is caused by a canker disease from an endemic fungal pathogen, *Quambalaria coyrecup* (1). It is hypothesised that *Phytophthora* species infect marri roots, predisposing trees to the canker disease. Hyperspectral remote sensing measurements, sensitive to leaf chemical and functional traits (especially related to foliar pigment and water content) are widely used to assess the condition of vegetation (2). This experiment examined the potential of hyperspectral measurements for early *Phytophthora* disease diagnosis in marri.

MATERIALS AND METHODS

Six-month-old seedlings were inoculated with three *Phytophthora* species. Half of the seedlings were flooded overnight every two weeks to induce zoospore formation. Three suites of measurements were used to monitor seedling stress responses. **Instantaneous plant function** was indicated by stomatal conductance measurements, using a portable photosynthesis system (CIRAS 2). **Short-term stress responses** mediated by pigment systems were detected with a hyperspectral spectrometer (ASD Fieldspec portable spectroradiometer) and **long-term structural effects** were determined from root and shoot biomass at harvest after three months.

RESULTS

Stomatal conductance was reduced by the flooding treatment, indicating lower photosynthetic rate, but was unaffected by the *Phytophthora* treatment (Figure 1).

The Vogelmann 3 spectral index (VOG3), a narrowband reflectance measurement sensitive for chlorophyll concentration indicated reduced chlorophyll content for the flooded seedlings but not for the *P. multivora 1* treatment (Figure 2). A decrease in top dry weight was observed with flooded seedlings ($p=0.06$) though *P. multivora 1* inoculated seedlings were unaffected by flooding (Figure 3).

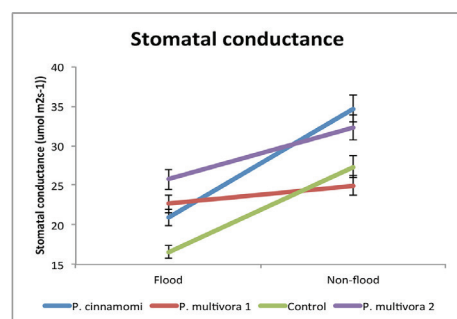


Figure 1. *Instantaneous plant function.* Interaction plot of the two-way effect of flooding and *Phytophthora* treatments on plant stress. Stomatal conductance was reduced by the flooding treatment.

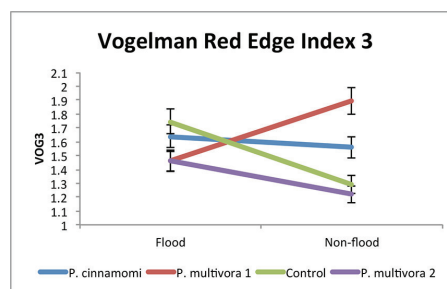


Figure 2. *Short-term stress responses* interaction plot. The VOG3-index provides an estimate of chlorophyll concentration, having a negative linear relationship to chlorophyll content. Reduced chlorophyll content was observed with the flooded seedlings but not for the *P. multivora 1* treatment.

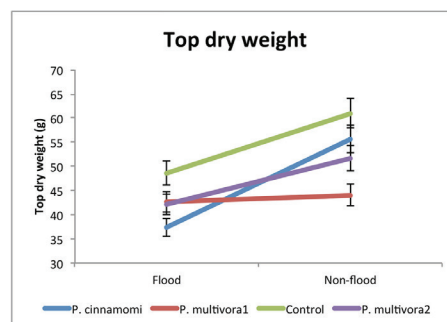


Figure 3. Interaction plot of *Long-term structural effect*. The top dry weight was reduced for all the flooded treatments, though the *P. multivora 1* treatment was unaffected by flooding.

DISCUSSION

Stomatal conductance and biomass revealed similar patterns: both photosynthesis and biomass were reduced by flooding but unaffected by *Phytophthora*. A similar trend was observed for chlorophyll concentration with flooding, but this was confounded by seedlings treated with *P. multivora 1*, which displayed the opposite response. The short course of the experiment (three months) could have been influential to the results.

Hyperspectral measurements display potential to detect stress (flooding, *Phytophthora*) in marri, though more experimental work needs to be done to determine if there are spectral signatures specific to *Phytophthora*-related stress in marri.

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POSTER 14

Direct DNA extraction from stromata decreases the time required for chestnut blight diagnosis

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INTRODUCTION

Chestnut blight, caused by the fungus *Cryphonectria parasitica*, is the most damaging disease of chestnut trees, including the species cultivated in Australia (*Castanea sativa*) which is highly susceptible. In 2010, chestnut blight was detected for the first time in Australia by Crop Health Services at DEDJTR. A delimitation survey and eradication program was deployed by the Victorian Biosecurity Division (BD). Symptoms of the disease on chestnut (cankers on trunks and branches, at times containing orange stromata) are easily confused with symptoms caused by the eucalyptus canker fungus, *Holocryphia eucalypti* (Fig. 1), which is far less damaging. Therefore, rapid and accurate laboratory diagnostic methods were a critical component of this response and continue to be employed in ongoing monitoring.

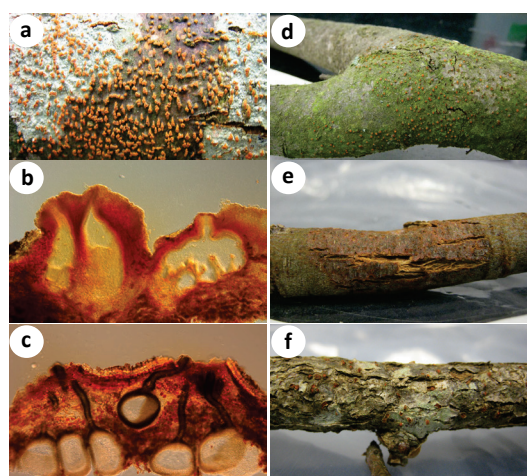


Figure 1. Orange stromata (conidiomata / ascostromata) of *Cryphonectria parasitica* (a-c) and *Holocryphia eucalypti* (d) on chestnut, and of *H. eucalypti* on eucalyptus (e) and oak (f).

The National Diagnostic Protocol for *Cryphonectria parasitica* (NDP 11) specifies isolation of the fungus into pure culture followed by DNA extraction and sequencing of the β -tubulin genes. The turn-around time from sample receipt until final conclusion can take 17 to 28 days using this method. There are no reliable species-specific primers to identify *C. parasitica*. To shorten the time for diagnosis, a method employing direct extraction from the symptomatic tissue was developed, bypassing the culturing and purification steps.

MATERIALS AND METHODS

Under a dissection microscope, clean stromatal tissues were obtained by removal of the outer layers using scalpels and forceps. The tissues then were subjected to direct DNA extraction using Qiagen DNeasy Plant Mini Kit. The β -tubulin region was amplified and sequenced using primers Bt1a and Bt1b (1). One to three stromatal samples were tested for each submitted diseased specimen, in addition to use of the recognised NDP 11 method. Of 11 specimens received by our laboratory in 2014, 20 stromatal samples were tested.

RESULTS AND DISCUSSION

Fifteen stromatal extracts (75%) provided enough DNA for a single round of PCR amplification to produce sufficient amplicons for downstream sequencing. Four stromatal extracts (20%) required a second round amplification of the PCR product using the same primers in a nested PCR in order to produce sufficient DNA amplicons for sequencing. The resulting sequences could clearly differentiate 4 isolates of *C. parasitica* from 7 isolates of *H. eucalypti* (6.4% difference; Fig. 2). By eliminating the requirement for fungal isolation into pure culture, the time for diagnosis was reduced to 48 hours for 95% of the suspect samples bearing orange stromata. The 5% of extractions that were unsuccessful (possibly due to contamination) demonstrated that several stromatal extractions per sample are required for a definitive diagnosis.

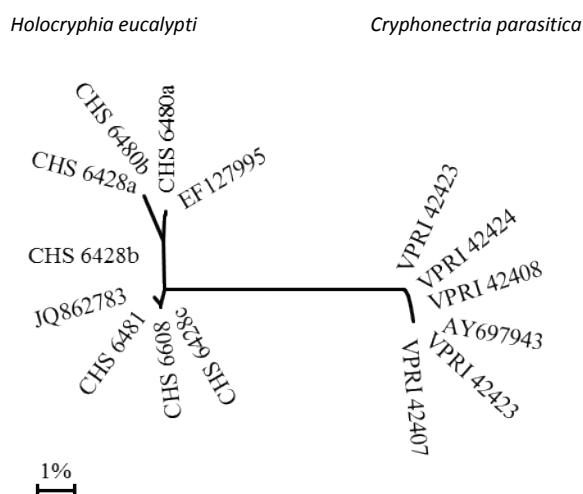


Figure 2. Species differentiation using β -tubulin gene sequencing for *Cryphonectria parasitica* (VPRI numbers) and *Holocryphia eucalypti* (CHS numbers). The GenBank accessions AY697943, EF127995 and JQ862783 were used as references. Bar shows the difference between the species.

This procedure resulted in a much more rapid diagnosis for incursion management purposes. However, fungal isolation and purification is still used to provide a specimen that can be VCG-tested and accessioned into VPRI for future reference.

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POSTER 15

A first step in the development of response curves to measure yield loss due to disease: a net form of net blotch case study

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INTRODUCTION

Production losses due to foliar and root diseases are a major financial constraint to the Australian wheat and barley industries. A national project has been formed to develop response curves that quantify the potential production losses that could be incurred as a result of such diseases on current Australian commercial wheat and barley varieties. These response curves will assist growers in making varietal selections and in deciding when intervention with fungicide applications may be necessary. The preliminary results from a net form of net blotch (NFB) experiment conducted in 2014 provide the first step toward the development of these response curves.

METHODS

Six commercial barley varieties (Compass, SY Rattler, Commander, Fairview, Henley and Charger) ranging in resistance from moderately resistant/moderately susceptible (MRMS) to very susceptible (VS) were tested under five NFB epidemics in a field experiment. The epidemics were established by applying varying quantities of diseased straw to the plots, along with timely applications of fungicide.

The experiment was arranged as a split-plot design with three replicates, whereby the six barley varieties formed main plots and the five NFB epidemics formed subplots.

Percentage leaf area diseased (% LAD) was assessed on the Flag leaf and three previous leaves (Flag-1, Flag-2 and Flag-3) of ten tillers randomly sampled from each plot, three times during the experiment. Yield and other grain quality traits were measured at the completion of the trial.

A linear mixed model was fitted to the yield and % LAD data separately, with variety, epidemic and their interaction as fixed effects and replicate, main plot and subplot as random effects. Additional random terms for sampling of tillers and leaves within tillers were included for the analysis of % LAD. Variance components were estimated using residual maximum likelihood using ASReml-R (1).

RESULTS

The five NFB epidemics resulted in significant yield differences within each of the six varieties (Fig. 1). This yield loss is best depicted in the most susceptible variety Charger, which can be seen to lose approximately 2.5t/ha from the least severe to most severe NFB epidemic.

LAD on the Flag-2 leaf chosen at the first measurement time (GS 71) demonstrates the range of epidemics achieved (Fig. 2).

DISCUSSION

This experiment shows that a range of yield responses can be measured across a range of established epidemics.

A statistical method for constructing yield response curves is currently under development. This method will use the Area Under the Disease Progress Curve (AUDPC) to model

the progression of the disease over the course of the experiment. A bivariate linear mixed model will then model yield against AUDPC to construct a response curve for each variety.

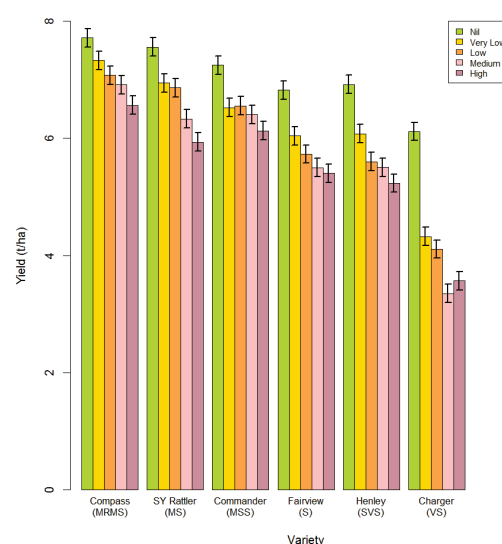


Figure 1. Yield (t/ha) for each variety by epidemic combination. Error bars denote standard error of the mean.

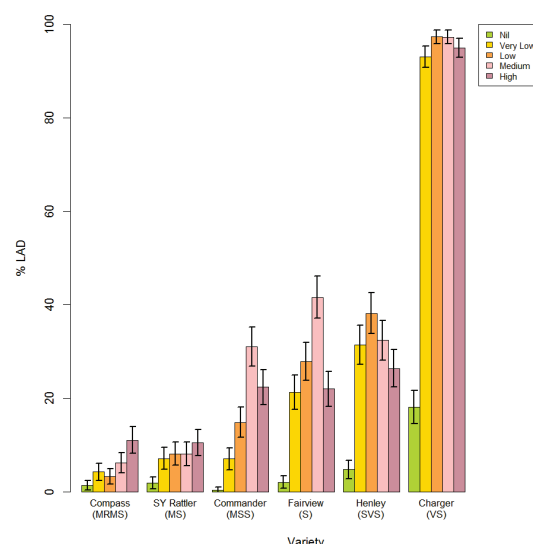


Figure 2. Percentage LAD on Flag-2 for each variety by epidemic combination. Varieties at GS 71 at first time of assessment. Error bars denote standard error of the mean.

ACKNOWLEDGEMENTS

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POSTER 16

Survival of *Phytophthora cinnamomi* in mine haul roadsW.A. Dunstan^A, T.I. Burgess^a, G.E.St.J. Hardy^A, V. Stokes^B^A Centre for *Phytophthora* Science & Management, VLS, Murdoch University, Murdoch, 6150 WA^B ALCOA of Australia, Huntly Mine, North Spur Rd., Pinjarra 6208 WA

INTRODUCTION

ALCOA mine and then restore 600 ha p.a of jarrah (*Eucalyptus marginata*) forest in south-western WA, extracting 23 Mt p.a. of bauxite ore for aluminium production. A significant proportion of mined areas are infested with *Phytophthora cinnamomi* and there is a considerable investment in management of operations in order to reduce spread of the pathogen. About 80 km of haul roads are known, or are likely, to be infested with *P. cinnamomi*. Roads and associated earth works are maintained vegetation free until restoration of mine pits has been completed, and are then in turn restored. It would be highly beneficial to restoration of many plant species if roads could be restored to a pathogen free status. As a preliminary to a larger study into the eradication of *P. cinnamomi*, we assessed the survival of *P. cinnamomi* in an artificially infested haul road.

MATERIALS AND METHODS

Because of high strength and bulk density of road materials, a tractor mounted vacuum drill was used to drill 48 mm diameter holes ($n = 600$) to depth of 50 cm into a 120 m section of decommissioned haul road. Autoclaved sections of Tagaste stems (ca. 2-3 cm long x 10-15 mm diameter) were inoculated with *P. cinnamomi*. After 10-11 weeks, colonised stems were enclosed in nylon mesh bags and buried in the drill holes (1 per hole, 50 cm deep), with the original excavated material, and to a similar bulk density. One hundred holes were drilled out and inoculum plugs recovered after eight and twelve months. Inoculum plugs were cut up and plated onto NARPH(1) medium (selective for *P. cinnamomi*). A subsample of the original non-sterile bagged inoculum that had been stored at 14-28°C was also assessed at twelve months.

RESULTS

Recovery rates of *P. cinnamomi* from both the haul road and the original inoculum are shown in Table 1.

Table 1. Recoveries of *Phytophthora cinnamomi* from inoculum plugs recovered from haul roads.

Inoculum source and age	% recovery
Road, 8 mths	26
Road, 12 mths	2
Lab, 12 mths (original inoculum)	64

DISCUSSION

Given that *Phytophthora cinnamomi* was recovered from 100% of inoculum plugs prior to their burial in the haul road, then there appears to have been a 98% decline in recoveries of *P. cinnamomi* from the road by twelve months. Soil temperature and moisture were monitored for most of the time that plugs were installed in the road. Temperature and/or soil moisture were unlikely to have effected survival of *P. cinnamomi* because, the soil temperature at 50 cm was never outside the upper limit (ca. 36°C) and lower limit (5-6°C) for growth(2). In contrast, at 10 cm, soil temperatures from the end of December

exceeded 36°C on 46 days, and exceeded 42°C (possibly lethal within a few hours) on 17 days. Soil moisture in summer to late autumn was never below 15% (volumetric).

The relatively good rate of recovery in non-sterile original inoculum, in contrast with road material (Table 1), suggests that other physical or chemical, or biotic factors, may have had a large effect on survival of *P. cinnamomi*. In any case, survival of *P. cinnamomi* (presumably as life stages mycelium and chlamydospores) appears to be very poor in an environment that was apparently almost free of potential plant hosts, where root material was detected in only 3 of the 600 drill holes. The results suggest that additional treatments may not be necessary in order to eliminate *P. cinnamomi* from haul roads, however this needs further testing with replication, and with other life stages of *P. cinnamomi* (in progress).

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POSTER 17

Application of biotechnology for nematode control in crop plants

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INTRODUCTION

Effective control of plant parasitic nematodes in crop plants will contribute hundreds of millions of dollars to global agriculture and help underpin future food security.

Recent advances now make it possible to exploit specific aspects of nematode-plant interactions to design strategies to reduce nematode invasion, impair nematode migration through tissues, prevent successful establishment or reduce feeding ability and reproduction. The knowledge of what genes are vital for successful nematode parasitism can also be used to develop new chemical control agents.

For transgenic technologies, delivery requires glasshouse and field testing, meeting OGTR regulations, adequate funding to complete statutory requirements, and public acceptance of GMOs.

The release of commercial crop varieties with new forms of nematode resistance, or new modes of delivery, is likely to become a commercial reality.

MATERIALS AND METHODS

Genomics. Genomic sequences are available for root-knot (RKN), cyst (CN) and other nematodes: we have generated transcriptomes for species of economic importance to Australian grains, eg the cereal CN nematode (*Heterodera avenae*) and the RLNs *Pratylenchus thornei* and *P. zeae*.

Gene silencing technology. We have applied gene silencing technology for control of RKNs, CNs and RLNs, by silencing target genes using dsRNA of target genes *in vitro* or via transgenic plants.

Effectors of RLNs. Comparative transcriptome studies have revealed potential effectors which RLNs use to invade host tissues and evade host defences.

RESULTS AND DISCUSSION

A comparison of putative parasitism genes in RKNs, CNs and RLNs is provided in Table 1. The significance of these results is that they help define which genes are required for feeding cell formation (RKN, CN), eg those listed as 'not identified' for RLNs, and those with common functions of migrating in plant tissues and evading host defences.

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Table 1. Parasitism gene	RKNs	CNs	RLNs	Putative or known function
Endoglucanases and precursor	+	+	+++	Hydrolysis of α 1,4-glucan
Pectate lyase	+	+	+++	Hydrolysis of α 1,4-linkages in pectate
Xylanase	+	Unknown	++	Hydrolysis of xylan
Expansin-like proteins	+	+	++	Cell wall softening or extension
Endo-1,3-beta-glucanase	Unknown	Unknown	++	Hydrolysis of β 1,3-glucan
Polygalacturonase	+	Unknown	++	Hydrolysis of α 1,4-D-galact. linkages
Arabinogalactan galactosidase/arabinase	+	+	++	Hydrolysis of pectin
Cellulose binding proteins	+	+	+++	Promote hydrolysis of crystalline cellulose
Thioredoxin	+	Unknown	+	Detoxification of ROS
Peroxioredoxin	+	+	+++	Detoxification of ROS
Superoxide dismutase	+	+	++	Detoxification of ROS
Glutathione-S-transferase	+		+++	Detoxification of ROS
Glutathione synthetase	+		+	Detoxification of ROS
Glutathione peroxidase	+	+	+++	Detoxification of ROS
SPRYSEC-RBP-1	+	+	+++	Suppression of host defenses
Sec-2/FAR	+	+	+++	Reduction in host defence response
Annexin	+	+	++	Protection of plant cells against stress
RANBP	Not present	+	+++	No known specific function in nematodes
14-3-3 and 14-3-3b	+	+	+++	No known specific function in nematodes
SKP-1	+	+	++	Ubiquitination, signal transduction
Ubiquitin extension protein	+	+	+++	Involved in ubiquitination
Calnexin/Calreticulin	+	+	+++	Calcium spiking
Beta-galactoside binding lectin (Galectin)	Unknown	+	++	No functional data available for nematodes
Aminopeptidase	+	+	+	Protein digestion/degradation
Proteases (e.g. serine, cysteine)	+	+	++	Protein digestion/degradation
Auxin	+	+	Not identified	Involved in giant cell formation
Cytokinin	+	+	Not identified	Activation of cell cycle
Peptide hormones (CEPs)	+		Not identified	Involved in giant cell formation
CLE peptides	+	+	Not identified	Mimic plant CLEs, functional evidence
16D10 CLE related peptide	+	Unknown	Not identified	Promotion of giant cell induction
Chorismate mutase	+	+	Unclear	Defence suppression, targeting SA pathway
NodL-like	+	Unknown	Not identified	No functional data available for nematodes
19C07	Unknown	+	Not identified	Modification of auxin influx in syncytium
10A06	Unclear	+	Not identified	Induction of antioxidant genes in syncytium
7E12	Unknown	+	Not identified	No functional data available
Transhyretin-like proteins	+	+	+++	Unknown function
Vap-1	+	+	++	Unknown function
SXP-RAL2	+	+	++	Unknown function

+= present; for RLNs:+++ in *P. thornei*, *P. coffeae* and *P. zeae* transcriptomes; ++ two of the three species; + in one species RLN

POSTER 18

Application of nanotechnology: a field trial to study the feasibility of a non-stick compound in preventing potential spread of *Phytophthora* infested soil through anthropogenic activities

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INTRODUCTION

The nanotechnology product, 'Ultra-Ever Dry', is used to coat an object and change it to a 'non-stick' surface so that it repels water and most materials including oil, mud, food, and paint. It has a commercial value in extending the life of a material treated with the product by preventing corrosion.

If the non-stick compound could be used in *Phytophthora* disease management, it would be cost-effective in the long run. Human activities such as walking and cycling through bush trails can inadvertently spread the soilborne pathogen. The infested soil sticking to footwear or bicycle tyres could be transported from one area to another and if the Ultra-Ever Dry product could stop mud sticking to these substrates, it could greatly contribute to maintaining plant hygiene.

The initial aim was limited to determining if 'Ultra-Ever Dry' could prevent mud sticking on shoes. If there were promising results, further research was to be considered.

MATERIALS AND METHOD

A pit (300 cm x 100 cm x 20 cm) filled with clay soil and watered to 30% moisture content was prepared to simulate a muck-filled bush trail (Fig 3). The Ultra-Ever Dry product with spray accessories (Fig. 2) was procured.



Fig 1. (left): Mud pit to simulate bush trail

Fig 2. (centre): Ultra-Ever Dry product

Fig 3. (right): Treated and non-treated shoe

Four pairs of working boots with three different leather uppers and sole types were used for the experiment. The shoes were cleaned with a hard brush and sprayed with the product as per product use recommendation. The shoes were worn and walked over the muck, following which their weight was taken to determine the amount of mud sticking on them.

The product was also tried on vehicle and bicycle tyres.

RESULT AND DISCUSSION

The non-stick property was clearly evident from the difference in the amount of mud sticking on the treated and non-treated shoes (Fig. 4). The overall differences notwithstanding, the amount of mud varied with the shoes of different brands and treads (Table. 1).

Table 1. Amount of mud (g) sticking on shoes treated and non-treated with the non-stick compound (Ultra-Ever Dry)

Weight of mud (g)		
	Treated	Non-treated
Shoes 1	62	146
Shoes 2	40	89
Shoes 3	158	278
Shoes 4	210	216



Fig 4. Treatment effect

Visual assessments for treatments on vehicle and bicycle indicated that the amount of mud on treated tyres (vehicle and bicycle) and mud-guard (vehicle) were less than non-treated ones.

In conclusion, it can be said that the product reduces the amount of mud sticking on the treated surface and may reduce the time for cleaning although it may not provide the level of prevention required for disease management. Further studies will be required to know whether the product would have any appreciable effect on time reduction for cleaning vehicles at wash-down stations.

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POSTER 19

Pinus radiata a new host for *Fusarium oxysporum* in Australia

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INTRODUCTION

In December 2013, wilt and high mortality of *Pinus radiata* seedlings were observed in the north of Perth, Western Australia (Fig 1). Symptomatic seedlings were sent to the Department of Agriculture and Food Western Australia, Plant Diseases Diagnostic lab, to identify cause of the disease. A *Fusarium* species was consistently isolated and identified on the basis of morphology, sequence analysis and a pathogenicity test.

MATERIALS AND METHODS

The symptomatic *Pinus radiata* seedlings were examined for root rot and vascular discoloration. Sections of the roots and stem tissues were separately surface-sterilised by immersion in a 1.25% aqueous solution of sodium hypochlorite for 2 min, rinsed in sterile water and dried in a laminar flow cabinet. The pieces were plated on half PDA and water agar plus antibiotics and incubated at 22±3°C for 7 days. Fungal colonies were subsequently sub-cultured onto PDA, then single-spored and plated on carnation leaf agar. A fungus with similar morphological characteristics was consistently isolated from roots and stem tissues.

Two representative isolates identified as *Fusarium* spp. were grown on PDA for a week at 25°C. Total genomic DNA was extracted from fungal mycelium with a DNeasy Plant Mini Kit (Qiagen, Melbourne, Vic., Australia) according to the manufacturer's instructions. PCR amplification was conducted and the internal transcribed spacer (ITS) region was amplified with primers ITS1 and ITS4 and part of the translation elongation factor 1- α (TEF) was amplified with primers EF1 and EF2. The polymerase chain reaction (PCR) products were sequenced and BLAST analyses used to compare sequences with those in GenBank.

A pathogenicity test was carried out on 5 month old *P. radiata* seedlings in a replicated glasshouse trial using a representative isolate. The seedlings were inoculated by dipping the roots in a spore suspension (1.0×10^6 spores/ml). Two weeks post inoculation disease symptoms were identical to those on naturally infected plants. Control plants remained asymptomatic (Fig 2).



Fig 1. Mortality and wilt of *Pinus radiata* seedlings caused by *Fusarium oxysporum*

RESULTS

All symptomatic *Pinus radiata* seedlings had root rot and vascular discoloration characteristic of fusarium symptoms were observed on the diseased seedlings. The cultural and morphological characteristics of the isolated fungus were similar to those described for *Fusarium oxysporum* Schlecht and this was confirmed by DNA sequence data. In a glasshouse inoculation test, seedlings had the same symptoms as those that were naturally infected and Koch's postulates were fulfilled by re-isolation of *F. oxysporum*. A culture of *F. oxysporum* was deposited in the Western Australia Plant Pathogen Collection (WAC13699).



Fig 2. *Pinus radiata* seedlings showing wilt symptom, two weeks after inoculation with *Fusarium oxysporum* (right) in comparison to healthy controls (left).

DISCUSSION

The *Fusarium oxysporum* species complex contains a large number of subspecies causing wilt and root rot diseases in a wide range of plant species worldwide. *Fusarium* species have been reported causing damping off, root rot and wilt of pine seedlings, but *F. oxysporum* has not been listed to be a pathogen on *P. radiata* in Australia (1). To our knowledge, this is the first report of *F. oxysporum* causing wilt and mortality on *Pinus radiata* in Australia. *F. circinatum* the causal agent of pitch canker is the most destructive disease of mature pines and nursery seedlings in America and South Africa. Incursion of *F. circinatum* is a great concern to many countries such as Australia and New Zealand (2).

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POSTER 20

Control of foliar diseases in Australian strawberry runner nurseries

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INTRODUCTION

Powdery mildew (caused by *Podosphaera aphanis*) and leaf blotch (caused by *Gnomoniopsis fructicola*) are important foliar diseases in Australian strawberry runner nurseries. *P. aphanis* and *G. fructicola* are also pathogens in fruit production, reducing fruit quality and marketable yield.

P. aphanis infects the leaves, flowers and fruit of strawberry. High humidity favours disease development. Dry weather and temperatures between 15°C and 27°C promotes spread. Powdery mildew symptoms include white, dense mycelial growth and chains of conidia giving a powdery appearance on the underside of leaves; irregular shaped yellow reddish-brown spots on the both upper and lower sides of the leaf; and leaf edges curled upwards. *G. fructicola* infects the leaves (leaf blotch), calyxes and fruit (stem end rot) of strawberry. Leaf blotch symptoms usually begin as circular, red-brown spots and develop into large areas of necrosis. The disease is favoured by warm humid conditions, but can still infect at low temperatures.

Mycobutanil, trifloxystrobin and penthiopyrad are registered for control of powdery mildew on strawberry. In 2014, quinoxifen was granted a permit for use in strawberry runner nurseries (non-fruiting). Apart from quinoxifen, the other active ingredients are not specifically registered to either nursery or fruit production. Due to the mechanisms of these fungicides against the pathogen, their repeated use at successive strawberry production stages may lead to the development of resistance in the fungus. There is currently no registered control for *G. fructicola* in strawberry.

This study evaluated the efficacy of several products for control of powdery mildew and leaf blotch.

MATERIALS AND METHODS

A series of screening trials were conducted in strawberry nurseries at Ballandean and Stanthorpe in Queensland and at three nurseries located in Toolangi, Victoria. Bare-rooted plants of 'Rubygem' in Queensland, and 'Albion' and 'Monterey' in Victoria, were planted to coincide with the 2013-14 nursery season. The plants were grown outdoors and under standard agronomy.

There were 12 treatments including a standard program for powdery mildew (sulfur, myclobutanil and trifloxystrobin alternated) and an untreated control (Table 1). Treatments were applied weekly using a knapsack sprayer. Application of treatment started in December/January and was completed in April/May.

The field experiments were laid out as randomised complete block designs. There were four blocks, and plots were 5 m in length with ten plants each.

Commercial runners were evaluated for the presence or absence of symptoms of powdery mildew and leaf blotch at the end of the trial period. The study relied on natural infection. One hundred leaves per plot were assessed in Queensland and fifty leaves per plot were assessed in Victoria, with the incidence expressed as a percentage of

affected leaves. Disease incidence percentages at each site were subjected to analysis of variance following arcsin transformation. Treatment means were back-transformed for presentation.

RESULTS

Bupirimate, cyflufenamid, quinoxifen, proquinazid, azoxystrobin + difenoconazole and the standard program were effective in trials in Queensland and Victorian strawberry nurseries against powdery mildew.

Azoxystrobin + difenoconazole, prochloraz and chlorothalonil were effective against leaf blotch.

Table 1. Effect of fungicide treatments from screening trials in Queensland and Victorian strawberry runner nurseries in 2013-14.

Treatments	Powdery Mildew % (Range) [*]	Leaf Blotch % (Range) [#]
Untreated	43 (30-60)	22 (7-31)
Sulfur	32 (22-44)	16 (4-21)
Standard Program	12 (8-19)	12 (4-14)
Quinoxifen	15 (12-19)	17 (4-26)
Proquinazid	16 (8-23)	15 (5-21)
Bupirimate	13 (7-18)	14 (5-21)
Cyflufenamid	10 (5-15)	17 (13-22)
Metrafenone	27 (17-33)	10 (2-16)
Azoxystrobin + difenoconazole	15 (11-19)	1 (0-3)
Chlorothalonil	29 (18-42)	8 (2-12)
Prochloraz	30 (21-40)	6 (0-11)
Spiroxamine	30 (14-48)	16 (5-22)

^{*} Averaged across four trials. [#] Averaged across three trials.

Disclaimer: Not all chemicals used in these experiments are currently registered for use on strawberry. Check current registrations for strawberries before using any of the chemicals. The product label is the official authority and should always be followed in relation to the use of a chemical.

DISCUSSION

The study identified effective products for control of powdery mildew and leaf blotch in strawberry nurseries. As a result, there are permit applications pending for products for use specifically in strawberry runner production (non-fruiting).

The inclusion of new chemicals and the development of a coordinated program between nursery and fruit production will have long-term benefits for the strawberry industry in Australia. New fungicides with different modes of action to the currently used fungicides will reduce the chance of fungi becoming resistant. This should extend the usefulness of all effective products and reduce disease incidence in nurseries and fruit farms.

ACKNOWLEDGEMENTS

This project has been funded by Horticulture Innovation Australia Limited with co-investment from Red Jewel Nursery, Sweets Strawberry Runners and the Toolangi Certified Strawberry Runner Growers Co-operative, and funds from the Australian Government. The Queensland Government has also co-funded the project through the Department of Agriculture and Fisheries.

POSTER 21

Western Australian antagonistic fungi reduce mycelial growth and sclerotia formation in *Sclerotinia sclerotiorum* isolated from canola

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INTRODUCTION

Sclerotinia sclerotiorum is a devastating plant pathogen attacking more than 400 plant species worldwide. Sclerotinia stem rot (SSR) disease caused by *S. sclerotiorum* can be a serious problem for canola (*Brassica napus* L.) production worldwide including in Western Australia (WA) with crop losses up to 40% in some years. Control of SSR disease in canola mainly relies on the use of fungicides; however some fungicides can be harmful for the environment and bio-ecosystem (1). Therefore, the use of biological control agents (BCAs) should be explored as one of the control strategies for managing SSR in WA. This research investigates the potential of local BCAs collected from canola growing areas across WA against SSR under controlled conditions.

MATERIALS AND METHODS

Isolate collection: Isolates of *S. sclerotiorum* were collected from diseased canola plants in the wheat belt of south-western WA. Isolates were obtained from sclerotia subcultured on Potato Dextrose Agar (PDA) and were then stored as mycelial cultures at 4°C. In addition, some potential biological control agents were opportunistically collected from southern agricultural regions of WA. The BCAs were isolated from canola paddocks and maintained in the laboratory as mycelial cultures at 4°C for further testing.

Screening of BCA's against SSR isolates: 15 different fungal BCA's were initially screened against an isolate of SSR (isolate 12) using dual culture tests. Isolate 12 was chosen as it showed rapid mycelial growth and was highly pathogenic. For dual culture tests, a mycelial plug (5 mm diameter) of each BCA isolate was incubated on PDA, about 1 cm from the edge of each petri dish. A mycelial plug of *S. sclerotiorum* removed from the colony margin of a 3-day-old culture grown on PDA was placed 6 cm away from the plug of the BCA isolate in the same petri dish. Controls were petri dishes similarly inoculated with just BCAs or *S. sclerotiorum* isolates alone. Plates were incubated in a 22°C growth room for 3 days, and were examined after 72 h for assessment of inhibition zones between BCAs and *S. sclerotiorum* isolates. At the end of the incubation period, radial growth was measured. Radial growth reduction was calculated in relation to growth of the control as follows: % Inhibition of radial mycelial growth = $[(C-T)/C] \times 100$, where C is the radial growth measurement of the pathogen in control plates, and T is the radial growth of the pathogen in presence of BCA. After two weeks of incubation, the number of sclerotia produced by *S. sclerotiorum* in each petri dish was determined. There were three replicate petri dishes for each treatment.

RESULTS

The percentage of growth inhibition of *S. sclerotiorum* by fungal BCAs ranged from 48% to 60% (Fig. 1). Furthermore, *in-vitro* study indicated that the number of sclerotia produced by the pathogen dropped significantly, ranging

from 0 to 10, compared with 28 in the untreated control (data not shown).

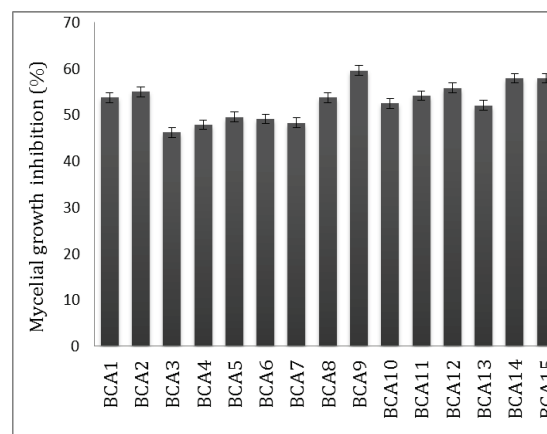


Figure 1. Percentage of mycelial growth inhibition of *S. sclerotiorum* in the presence of potential WA fungal biological control agents

DISCUSSION

Current investigations indicate that all tested BCA fungal isolates have potential for inhibiting mycelial growth of *S. sclerotiorum* as well as reducing the number of sclerotia produced by the pathogen *in vitro*. However, the mode of action of BCA's in inhibiting the growth of *S. sclerotiorum* is unknown. One explanation of this mycelial growth inhibition may be that the BCAs compete with the pathogen for nutrients; however, further work is needed to determine the influence of other modes of action such as myco-parasitism and antibiosis. Research is in progress to identify the species of each fungal BCA and to evaluate the effectiveness of the BCA isolates in managing *Sclerotinia* stem rot under controlled and field conditions.

ACKNOWLEDGEMENTS

We thank the Australian Centre for International Agricultural Research (ACIAR) for support through their John Allwright PhD Fellowship program at Murdoch University to the first author. Many thanks to Aberra of DAFWA for technical assistance.

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POSTER 22

Distribution of *Sclerotinia sclerotiorum* sclerotes in Western Australia in 2013

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INTRODUCTION

The delivery levels in grain of sclerotes, the resting bodies of *Sclerotinia sclerotiorum* were used as a method of assessing the incidence and severity of sclerotinia stem rot (SSR) in canola in different regions and rainfall zones of the West Australian (WA) grain belt. Sclerotes are formed in and on canola plants and taken up with the grain during the harvest process.

MATERIALS AND METHODS

In WA approximately 95% of grain is delivered into the grower owned Cooperative Bulk Handling (CBH) system. CBH offers a limited release of data when it is in the interest of industry and in this context CBH made 2013/14 harvest data on sclerote contaminated canola grain available for this study.

Canola delivered to CBH at harvest must comply with a set of standards that are aligned with the Grain Trade Australia (GTA) specifications. The current standards allow canola grain to have maximum concentration of 0.5% of sclerotes by weight in a representative 500 g sample. The minimum detection level is 0.01% (50 mg). No canola was refused delivery for exceeding the sclerote limit alone.

The regional areas were; North = canola grain receipt points north of Wongan Hills; Central = receipt points between Wongan Hills and Lake Grace; South = receipt points south of Narrakine and Lake Grace.

RESULTS

Of the 1.796 million tonnes (1) of canola grain delivered into the CBH system in the WA harvest of 2013/14, sclerotes were detected in 3.9% or 70,000 t.

Sclerotes were present in affected grain across the full range of detection from 0.01 to 0.49%. The distribution of affected grain shows that most contained sclerotes at the lowest levels of detection - 0.01 percent (32%) and 0.02 percent (23%). Ninety percent of grain had a sclerote concentration of 0.09 percent or less. Figure 1 shows the regional breakdown of the affected grain at each sclerote level. Grain in the upper range of sclerotes (0.31 – 0.49%) represented less than one per cent of the affected grain.

The majority of affected grain (71%) was grown in the high rainfall zones (>450 mm annually) with 29% from the medium rainfall zones (350 – 450 mm) and < 1% from the low rainfall zones.

The Southern region produced 63% of the sclerote contaminated grain (5% of total regional production), the Central region 12% (1% of regional production) and the North 25% (7% of regional production). The Northern region produced most of the grain that had sclerotes present at higher concentrations (Figure 2).

DISCUSSION

SSR is prevalent in the high rainfall area which produces much of the canola in WA. Medium rainfall areas had significantly less sclerote affected grain delivered while the low rainfall had negligible levels. The southern region

produced most of the affected grain which is unsurprising given that most of the land in this region is within the high and medium rainfall zones. The northern region had the highest proportion of its canola crop affected and the sclerote level in grain was higher.

Across WA 2013 was a season conducive to SSR, however the sclerote levels in grain remained relatively low.

Analysis of receipts site by site would alert growers to hot spots where SSR is worst, highlighting the need for treatment of affected crops.

One limitation of this study is that grain with high levels of sclerotes may have been graded before delivery to remove them.

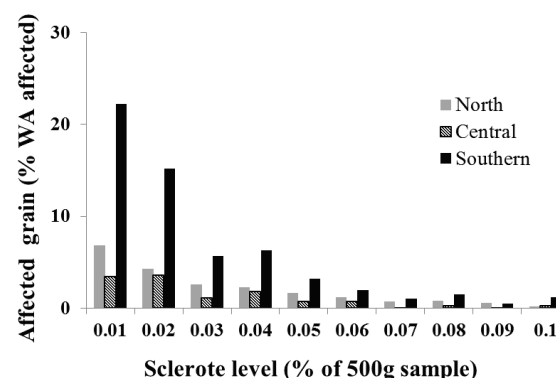


Figure 1. Amount of sclerote affected grain (%) from each region (North, Central and South) by sclerote concentration (% 500g sample) for the WA grain belt in 2013. Only the data from levels which had 0.01% or less sclerotes is shown which represents 90% of all the affected grain delivered.

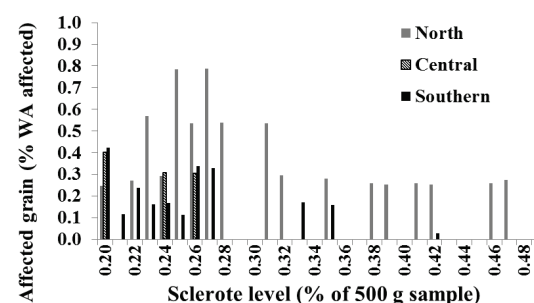


Figure 2. Percentage of sclerote affected canola grain with 0.2% or more sclerotes present for the North, Central and South regions of the WA grain belt in 2013.

ACKNOWLEDGEMENTS

CBH Group provided the grain delivery data used in this study. DAFWA and GRDC jointly fund the project DAW00229 – Integrated disease and pest management.

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POSTER 23

Yield loss to *Fusarium* crown rot: is there a better choice among barley and wheat varieties?D. Hüberli^A, M. Connor^A and K. Gajda^A^A Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court, South Perth, 6151, WA

INTRODUCTION

Fusarium crown rot, caused predominately by the stubble-borne fungus *Fusarium pseudograminearum*, is one of the major root and crown disease constraints on cereal production in Australia. In 2009 it was estimated to cost Australian grain growers \$97 million annually in wheat and barley (1, 2). WA's losses to this disease were estimated at \$7 million which may be largely underestimated. In 2014, many growing regions in WA were impacted by crown rot, exacerbated by dry weather conditions during grain fill. For example, reports from Merredin indicated that crown rot affected 30-50% of wheat paddocks.

Several new wheat varieties have been released recently with improved tolerance to crown rot. No experimental field evidence is currently available to grain growers of the effect of crown rot on variety yields in WA. Hence, there is an on-going need to evaluate wheat and barley varieties to demonstrate to growers the economic benefits of adoption of varietal selection in paddocks with high crown rot pressure.

MATERIALS AND METHODS

Barley and wheat field experiments were sown at Merredin and Wongan Hills Research Stations on 28 May and 4 June in 2014 and will be repeated in 2015 and 2016. Each experiment had 12 varieties in paired plots, plus/minus *F. pseudograminearum* inoculum, with 4 replications. At Wongan Hills no crown rot DNA was detected in the soil, while at Merredin a high level of background inoculum resulted in significant infection in uninoculated plots. Data presented here is from Wongan Hills. Grain yield (t/ha) and level of disease as the number of white heads were measured.

RESULTS

All barley and wheat varieties had some level of disease and yield reductions (Figure 1 and 2) in inoculated plots with significant differences evident between varieties. In barley, Litmus, LaTrobe and Baudin had the lowest yield reduction from crown rot inoculation, with Litmus being the highest yielding variety in presence of crown rot (Figure 2). A similar trend in variety yield response was observed at Merredin.

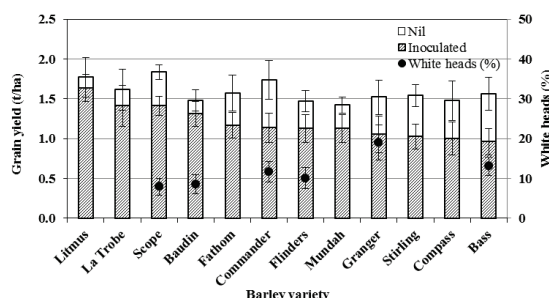


Figure 1. Grain yield (12 varieties) and white head incidence (6 varieties) for barley in nil and *Fusarium pseudograminearum* inoculated plots at Wongan Hills in 2014. Crown rot resistance rankings are not available.

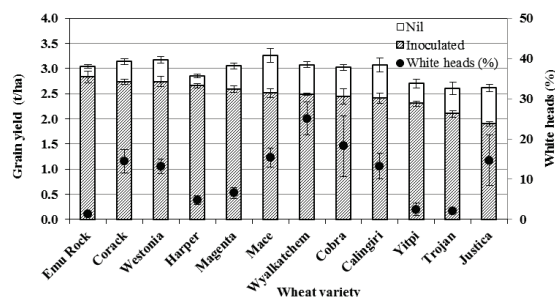


Figure 2. Grain yield and white head incidence for 12 wheat varieties in nil and *Fusarium pseudograminearum* inoculated plots at Wongan Hills in 2014. Resistance rankings for Emu Rock and Trojan are moderately susceptible (MS), Magenta is MS to susceptible (MSS), and remaining varieties are susceptible to crown rot.

In wheat, Emu Rock had the highest yield and the lowest white head expression in crown rot affected plots and also had the least reduction in yield from crown rot infection. More than 500 kg/ha yield loss and elevated disease expression occurred in Justica, Mace, Wyalkatchem, Cobra and Calingiri. At Merredin, Emu Rock was also the best performing variety.

DISCUSSION

This is the first report of inoculated crown rot field experiments to evaluate infection and yield loss in barley and wheat varieties in WA. All varieties of barley or wheat were found to be affected by the disease and all had some level of yield reduction, however, significant differences between varieties were evident.

The results show that variety choice under crown rot inoculum can have an impact on yield. For example, under crown rot inoculum, Emu Rock yielded 300 kg/ha more than Mace, the variety of choice in 60% of the cropping area of WA in 2014. However, in the plots without crown rot, Mace out-yielded Emu Rock by 200 kg/ha. These preliminary results indicate that understanding the crown rot disease history of a paddock and choosing varieties with appropriate disease resistance ranking can influence crop yield performance.

ACKNOWLEDGEMENTS

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POSTER 24

New Zealand post-entry plant quarantine services

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New Zealand's agricultural and horticultural industries depend on the introduction of new plant material to remain globally competitive with respect to accessing export markets. Plant material entering New Zealand may be subject to Post-Entry Quarantine (PEQ) to ensure unwanted pests and diseases are not inadvertently imported. Some of these could cause significant damage to our plant-based industries and the environment if they were to become established.

There are three levels of PEQ which are determined by the level of the pest and disease risk associated with the imported plant material; Level 3 being the highest level of quarantine. During the PEQ period, plants are inspected at regular intervals for pests and disease symptoms. Samples from plants suspected of harboring unwanted pests and diseases are sent to an approved diagnostic laboratory for general diagnostic testing. In addition, plants may have to undergo a strict testing regime, regardless of whether they show symptoms; this testing regime is specified in the relevant import health standard (IHS) for each plant species. On completion of the final inspection, and if the plants are found to be free of unwanted pests and diseases, biosecurity clearance is issued for release of the imported plants from PEQ. Importers are then free to use and distribute the new material within New Zealand.

The Plant Health and Environment Laboratory (PHEL) of the Ministry for Primary Industries (MPI), New Zealand runs a Level 3 glasshouse and tissue culture facility. The laboratory, which is accredited to ISO 17025, also has the capacity to carry out the full range of quarantine tests as specified in the IHS for all Level 3 crop species. Scientists in the Virology and PEQ team at the PHEL produce PEQ testing manuals which help PEQ operators interpret the IHS requirements more easily. Manuals have been written for most of New Zealand's important crops and are publicly available on the MPI website. They can be used by anyone providing Level 3 quarantine testing within New Zealand. During the last three years, PHEL has provided quarantine space and/or testing for a range of crops including Citrus, Fragaria, Ipomoea, Malus, Prunus, Pyrus, Rubus, Solanum, Vaccinium and Vitis.

POSTER 25

Resistance-related responses in model and native plant species infected with *Phytophthora cinnamomi*

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INTRODUCTION

Phytophthora cinnamomi is an oomycete plant pathogen which causes root rot disease in both natural and agricultural systems worldwide. The complete eradication of the pathogen is not possible at the landscape scale, thus, disease management needs to be focused on suppressive chemicals and development of resistant populations (1). However, suppressive chemicals are not a long term solution as the pathogen may develop resistance and chemicals may present toxicity to the plants or other undesirable effects on the ecosystem. Hence, an understanding of the key molecular responses employed by resistant species is necessary to develop durable control measures. *P. cinnamomi* exhibits a large host range and only a small number of plants are highly resistant to this pathogen. So, the key questions of why, and by what mechanisms, some species are able to survive infestation remain unanswered. Therefore, the current study is investigating the resistance mechanisms that *Zea mays* (a resistant model plant) and *Lomandra longifolia* (a resistant Australian native plant) engage to fight *P. cinnamomi*.

MATERIALS AND METHODS

Zea mays (Variety: Early Leaming), *Lupinus angustifolius* and *Lomandra longifolia* were grown in a soil free plant growth system and roots were inoculated with zoospores (1×10^5 spores/mL). Lesion lengths were measured every 24 hours after inoculation until 168 hours. To analyse candidate gene expression in the model resistant plant *Z. mays*, roots were inoculated and then harvested at different hours post inoculation (hpi). Total RNA was extracted from harvested roots and cDNA was synthesised. Semi-quantitative and quantitative PCR was conducted to analyse the expression of candidate genes (2).

RESULTS AND DISCUSSION

Lesion development and root changes produced by the interaction between *P. cinnamomi* and the Australian native plant *L. longifolia* were observed under laboratory conditions. Developments of lesions on a highly susceptible non-native plant, *L. angustifolius*, were recorded for comparison. The lesion length on roots of *L. longifolia* was significantly smaller than in *L. angustifolius*. Moreover, the lesions on *L. longifolia* roots were restricted to a small area whereas they expanded rapidly on susceptible species over time (Figure 1). We wish to investigate the genes that are involved in resistance in *L. longifolia* and are using the model *Z. mays* system to provide leads into monocot resistance.

To identify resistance related genes in *Z. mays* a microarray analysis had been previously performed and a variety of genes were found to be up-regulated (2). The current study confirmed the up-regulation of a number of these genes over a comprehensive early response time course. The higher expression of several genes of interest was found by 3 hpi compared to controls and the expression gradually increased up to 12 hpi (Figure 2).

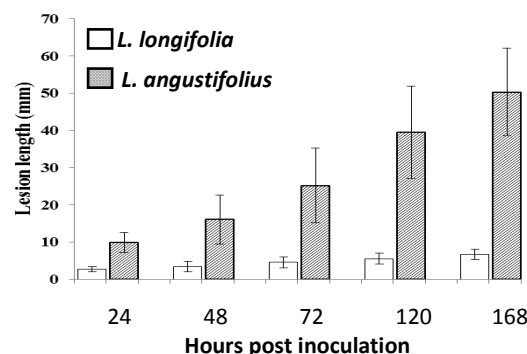


Figure 1. Root lesion length of *L. Longifolia* and *L. angustifolius* following inoculation with *P. cinnamomi*. Mean value was calculated from 60 plants per species. Error bar indicates standard error calculated from replicates.

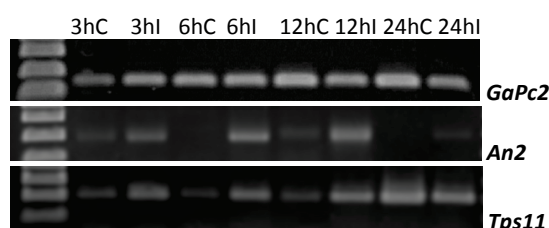


Figure 2. Expression analysis of resistance-related genes in *Z. mays* following inoculation with *P. cinnamomi*. The expression levels of *An2* and *Tps11* were up-regulated following inoculation at different hpi. *GaPc2* was used as an internal control. hC= hours post inoculation control and hI= hours post inoculation by *P. cinnamomi*.

CONCLUSION

L. longifolia showed a high level of resistance to *P. cinnamomi* that was related to lesion restriction. Resistance related genes are upregulated following infection of *Z. mays* roots with the pathogen. Future work will investigate resistance components in *L. longifolia*.

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POSTER 26

Evaluation of correlation methods for co-occurrence network construction of rice crop health survey data

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INTRODUCTION

On-farm surveys are useful sources of data to help understand yield reduction factors in farmers' fields. These data also help us understand the interactions and importance of pests and the complex relationships within agroecosystems.

Network analysis is a promising tool frequently used to describe the pairwise relationships of a large number of variables. Network analysis of the co-occurrence patterns of pest and disease could offer new insight into pest management. Since different correlation measures lead to structurally different co-occurrence networks and provide different information, selecting the correct correlation method is critical.

MATERIALS AND METHODS

Survey data collected from 2010 to 2012 in 420 farmers' fields in irrigated lowland rice-growing areas of five Asian countries (India, Indonesia, Philippines, Thailand and Vietnam) were based on 'A survey portfolio to characterise yield-reducing factors in rice' (1).

An incidence matrix was generated to hold the observations in R (2). Pairwise scores between variables were computed using Pearson, Spearman, Kendal and biweight midcorrelation measures. Network models were visualised using the 'qgraph' package for R (3).

RESULTS AND DISCUSSION

An evaluation of the four correlation measures on the basis of their weights of edges (correlation coefficients) shows two groups clustered according to hierarchical clustering using Euclidean distance. The Spearman and Kendall correlations were grouped in rank-based methods; the other group, Pearson, and biweight midcorrelation, were non-rank-based correlations.

A Shapiro-Wilk test indicated that the data were not normally distributed with many outliers, thus non-rank-based methods were unsuitable. The Spearman correlation measure was selected because of its robustness to noise, outliers, and ability to accurately predict the interactions in the network versus the Kendall correlation measure.

A weighted co-occurrence network analysis with cutoff on p-value of 0.05 revealed that brown spot is the most central connection (Figure 1). This indicates that controlling brown spot would imply control for connected yield reducers. Bacterial leaf blight, sheath rot, sheath blight, red stripe, and silver shoot formed a cluster, which indicates that these variables occurred simultaneously.

Appropriate correlation measures should closely associate with prior knowledge of the biological system. In this study, Spearman's correlation was found to be the most suitable measure as supported by the results that accurately

captured a known relationship between brown planthopper and whitebacked planthopper (4).

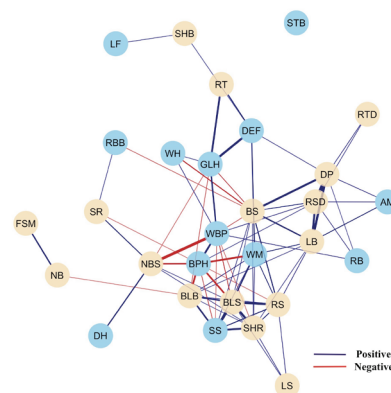


Figure 1. Co-occurrence network of crop health survey data based on Spearman correlation. Blue and brown nodes represent pest injuries and diseases, respectively. The layout of the network graph is based on the Fruchterman-Reingold algorithm, which places nodes with stronger or more connections closer to each other. AM: army worm, BLB: bacterial leaf blight, BLS: bacterial leaf streak, BPH: brown planthopper, BS: brown spot, DEF: defoliator, DH: deadheart, DP: dirty panicle, FSM: false smut, GLH: green leafhopper, LB: leaf blast, LF: leaf folder, LS: leaf scald, NB: neck blast, NBS: narrow brown spot, RB: rice bug, RBB: rice black bug, RS: red stripe, RSD: ragged stunt disease, RT: rat, RTD: rice tungro disease, SHR: sheath rot, SHB: sheath blight, SR: stem rot, SS: silver shoot, STB: stink bug, WBP: whitebacked planthopper, WH: whitehead, WM: whorl maggot.

ACKNOWLEDGEMENTS

Syngenta provided the financial support and collected the on-farm survey data as part of the SKEP collaboration.

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POSTER 27

Interaction of fungicide and genetic resistance for the management of barley leaf rust

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INTRODUCTION

Barley leaf rust caused by *Puccinia hordei* G. Otth has become an issue in recent years in the south coast of Western Australia (WA) mainly due to a lack of phenotypic resistance in widely grown malting barley varieties and a conducive environment in the region.

Varieties with major gene resistance are race specific and not durable. For example in 2013, the barley variety Bass which possesses *Rph3* gene (major gene) lost resistance to the leaf rust pathogen within a year after the variety was released. However, varieties with additive minor genes possess durable resistance, commonly known as Adult Plant Resistance (APR) and are race non-specific. The expression of APR in a variety varies from tillering (Z20) to full head emergence growth stage (Z59) and is primarily influenced by temperature and other factors (Park R, pers. comm.)

The aim of this study is to determine how some of the new varieties with APR perform in the south coast environment of WA and respond to fungicides that control leaf rust.

MATERIALS AND METHODS

Experiments were conducted in 2014, near Wellstead (Latitude -34.572724° and Longitude 118.409512°). Six barley varieties which possess a range of leaf rust resistance genes (Fig.1a) were sown at 75 kg/ha on 25 May using a 6 row cone seeder with knife points and harrows on to 2013 canola stubble. Plots were 15 m by 1.8 m in a Latinised split –plot design with 3 replications.

Fungicide Cogitto[®] (active Propiconazole + Tebuconazole) was applied at 125 mL in 80L water/ha during early tillering growth stage (Z24, time 1 {T1}) on 17 July and subsequent foliar spray, Prosaro[®] 420SC (active Prothioconazole + Tebuconazole) was applied at 150 mL in 80L water/ha with 1% v/v Hasten 4 weeks later during early stem elongation growth stage (Z32, time 2 {T2}) on 15 August.

Trial was assessed for disease of all open leaves at bi weekly intervals. GenStat 16th Edition was used to analyse data. To reduce the variance in the % leaf area diseased, the data was analysed after angular transformation.

Since the trial site was heavily affected with Rhizoctonia, yield was assessments by 3 X 1 square metre quadrat cuts per plot.

RESULTS AND DISCUSSIONS

The leaf rust pressure was moderate at the trial site. At ear emergence (Z55), all barley varieties started to show leaf rust. Bass and La Trobe untreated plots showed greatest levels of leaf rust followed by Flinders, GrangeR, Shepherd and Westminster (Fig. 1a). There were no significant treatment differences among varieties such as Flinders, GrangeR, Shepherd and Westminster. Also there were no significant treatment differences between single application of foliar fungicide and untreated on Bass and La Trobe. Two foliar sprays significantly reduced leaf rust compared to untreated and single foliar spray on Bass. In La Trobe, there was no significant foliar fungicide treatment difference

between untreated vs single spray or between single spray vs two sprays (Fig. 1a).

The yield for untreated plots varied from 0.7 t/ha (Bass) to 5.6 t/ha (GrangeR), as shown in Fig. 1b. Application of foliar fungicides significantly increased the yield on Bass and La Trobe but treatment differences on yield were not significant on other varieties.

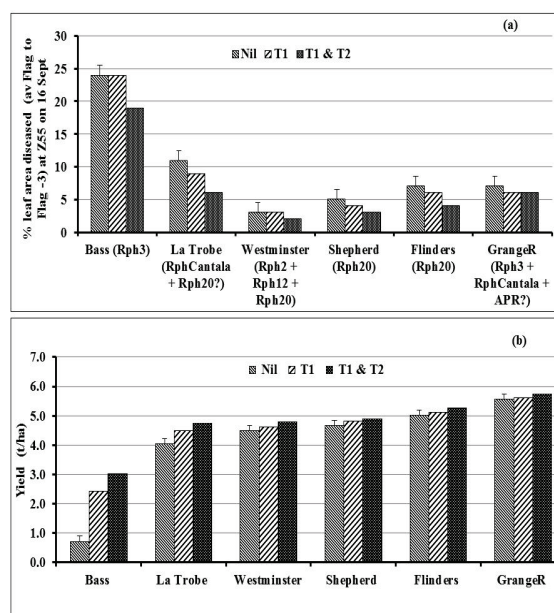


Figure 1. Effect of fungicide treatments on leaf rust control (a), and grain yields (b) of six barley varieties with different resistance profiles at Wellstead WA in 2014.

Screenings and protein were high and grain brightness was low in Bass, Flinders, GrangeR, La Trobe and Westminster and therefore did not meet malting quality standards (data not shown). This may be due to combined effects of lack of soil moisture during the grain filling and to some extent disease pressure. Shepherd is feed barley.

ACKNOWLEDGEMENTS

The authors acknowledge the trial site provided by Mark and Pamela Wood and financial assistance from Grains Research and Development Corporation, DAW00229.

POSTER 28

Understanding the barley leaf rust new pathotype 5457 P- in Western Australia

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INTRODUCTION

Leaf rust of barley (*Hordeum vulgare*) caused by *Puccinia hordei* Oth is an issue for barley production in the southern region of Western Australia (WA). The new pathotype occurrence in the region can be mainly due to a single step mutation of the existing pathotype such as 5453P-, sexual recombination via alternate host *Ornithogalum* spp. or introduction from outside (Robert Park pers. comm.).

In 2013, the *Rph3* gene for leaf rust resistance in the barley variety Bass was rapidly broken down within a year of its release.

Understanding the pathogenic variability of the new pathotype arising from an alternate host is of great interest in order to formulate management options.

MATERIALS AND METHODS

Teliospore germination and inoculation of alternate host. Telia originated from barley variety Bass in South Stirling of WA were used to induce teliospore germination and basidiospore production, and subsequently inoculated *O. eigii* plants in the greenhouse. Arising pycnia were cross fertilised resulting aecia. Aeciospores were transferred to barley varieties Manchuria and Estate, method described by (1).

Determination of virulence using differential set. Eighteen single aecial clusters originating from 3 different *O. eigii* plants were inoculated on differential set (25 lines/varieties which possess different rust resistance genes) and raised in the glasshouse as previously described (2). Inoculated plants were assessed 10 to 14 days post-inoculation and the infection type was recorded on a scale of 0 to 4 (3); a score of 0 to 2 class is classed as resistant and a score of 3 to 4 is classed as susceptible.



Figure 1. *P. hordei*, WA isolate 22628 on the primary (A) and alternate (B) hosts. A) Barley variety Estate showing uredinia and subsequent telia formation on 1 to 2 leaf stage. B) Pycnia (marked for cross fertilisation) on *O. eigii* and single aecial cluster with a few pycnia at the centre (inset). Plants were artificially inoculated in the greenhouse (21±2°C).

RESULTS AND DISCUSSION

Table 1. Response of *Hordeum vulgare* genotypes with different resistance genes to single aecial clusters originated from three different *O. eigii* plants infected with the WA isolate 22628.

Tested Line	Resistance gene(s)	22628-1						22628-2						22628-3					
		ε379	ε388	ε389	ε390	ε391	ε392	ε380	ε383	ε384	ε385	ε386	ε387	ε394	ε395	ε396	ε397	ε398	
1/Gun	Sue	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
2/Sudan	Rph 1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3/Berg	Rph 1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
4/Peruvian	Rph 2	S	S	S	S	S	S	YR	YR	S	S	S	S	S	YR	YR	S	S	
5/Reka 1	Rph 2-P	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
6/Ricardo	Rph 2-7	S	S	R	S	R	S	YR	YR	R	R	R	YR	S	S	S	S	S	
7/Estate	Rph 3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
8/Gold	Rph 4	S	S	S	S	S	YR	YR	S	S	S	S	S	S	S	R	S	S	
9/Rph 13	Rph 13	R	R	R	R	R	R	YR	R	R	R	R	R	R	R	R	R	R	
10/Quinn	Rph 2-5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
11/Magall 104	Rph 5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
12/Bolivia	Rph 2-6	S	S	S	S	S	S	S	S	S	S	S	S	YR	YR	S	S	S	
13/C. Capa	Rph 7	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
14/Rph 14	Rph 14	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
15/Egypt 4	Rph 8	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
16/Ag	Sue	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
17/Rph 10	Rph 10	S	S	S	S	S	S	R	YR	S	S	S	S	S	S	YR	R	S	
18/Rph 11	Rph 11	S	S	S	YR	S	S	R	YR	S	S	S	S	S	S	YR	S	S	
19/Triumph	Rph 12	S	S	S	S	S	S	R	S	S	S	S	S	S	YR	S	YR	YR	
20/Prize	Rph 19	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
21/Cutter	Rph 19	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
22/Q21861	Rph Q	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
23/Cantala	Rph C	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
24/Calleachini	Rph X	S	S	S	YR	S	S	S	S	S	S	S	S	S	S	S	S	S	
25/Steff. new	Rph 9 Hor 15	YR	S	S	YR	S	S	YR	YR	S	S	S	S	S	YR	YR	YR	YR	
ADM		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
MANCHURIA		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	

Virulence of isolates collected from alternate host. All 18 isolates originated from the alternate host showed virulence on differentials: Sudan; Estate; Cantala and Line Q21861. These 18 isolates are similar also in the avirulent response on 10 other differentials, which points out of a ‘newcomer’ isolate other than a result of a single step mutation.

Interestingly, unlike Sudan which only possess the *Rph1* gene the results suggests that the Berg variety possess *Rph1* and additional unknown resistance genes.

Variety Estate which possess *Rph3* gene was susceptible to the WA isolates as shown in Table 1. This suggests that the 5457P- pathotype carries a recessive homozygous gene for virulence on *Rph3*.

Previously, it was reported that *Rph7*, *Rph11*, *Rph14*, *Rph15*, *Rph18* and *Rph21* are the only major resistance genes available for incorporation into Australian barley varieties (Robert Park pers. comm.). However, the most notable finding of the current study is the early detection of virulence to *Rph11*. Conveying this information to breeders in advance is pivotal in order to prevent the introduction of *Rph11* containing lines to our region due to the possible rapid resistance breakdown of lines containing *Rph11*.

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POSTER 29

Application of quantitative precipitation model to improve spatial resolution of plant disease forecast models

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INTRODUCTION

Rainfall is an essential driving factor for many plant disease forecast models. Rainfall estimation is necessary to enhance the spatial resolution of plant disease forecast. Because of spatial characteristics of rainfall events, however, spatial interpolation of rainfall data often results in erroneous estimations for locations where observed data are not available. We have found that spatial interpolation of rainfall data using the inverse distance weighting (IDW) did not represent the actual spatial patterns in the map image of rainfall data. To improve quality of rainfall data at high spatial resolution, the quantitative precipitation model (QPM) was applied in this study as an alternative to IDW. QPM generates grid rainfall data at the spatial resolution of 1km × 1km using upper atmospheric weather data measured by radiosondes and hourly rainfall data measured by AWS at 634 locations.

MATERIALS AND METHODS

Weather data processing: Hourly rainfall data monitored by automated weather stations (AWS) at 634 locations in S. Korea were used to estimate grid rainfall data at the spatial resolution of 1km × 1km using IDW and QPM. Of 711,504 intersections in the grid map of S. Korea, 52 intersections were overlapped with the sites of AWS. Rainfall data estimated by IDW and QPM for the 52 overlapped sites were compared with the observed rainfall by AWS. The data covered 31 days (Jul. 15-Aug. 14) and 56 days (Jul. 2-Aug. 26) in 2013 and 2014, respectively.

Performance of IDW and QPM: Performance of IDW and QPM was evaluated by comparing with observed rainfall data in three ways: spatial map image comparison, contingency table analysis based on bias score, and regression analysis between observed and estimated rainfall data. Hourly infection periods of rice blast estimated by the Yoshino model based on weather data observed by AWS and estimated by IDW and QPM were compared.

RESULTS

Table 1. Contingency table of hourly rainfall estimations by QPM and IDW as compared with rainfall observed by AWS for overlapped locations in 2013 and 2014.

Index ^a	7.15-8.14, 2013		7.2-8.26, 2014	
	QPM	IDW	QPM	IDW
Hit	1306	2420	5960	8014
Miss	412	250	2452	398
False alarm	535	6754	1020	13075
Correct negative	36435	29264	56424	44369
Bias score	1.07	3.44	0.83	2.51
POD	76.0%	90.6%	70.9%	95.3%
FAR	29.1%	73.6%	14.6%	62.0%

^a Hit=event forecast to occur, and did occur; Miss=event forecast not to occur, but did occur; False alarm=event forecast to occur, but did not occur; Correct negative =event forecast not to occur, and did not occur; Bias score=(False alarm+Hit)/(Miss+Hit); Probability of detection (POD)=Hit/(Hit+Miss); False alarm ratio (FAR) =False alarm/(Hit+False alarm).

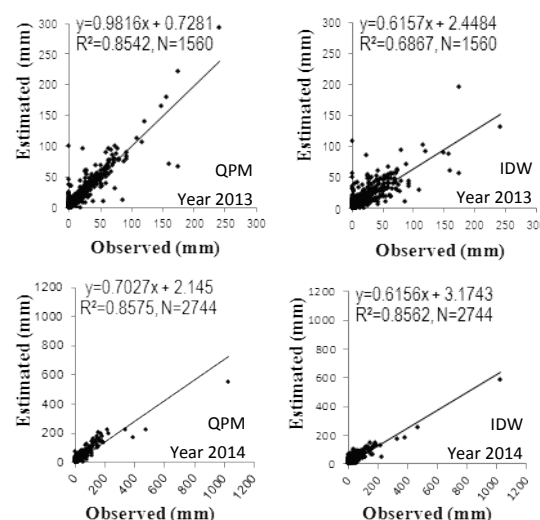


Figure 1. Regression analysis between daily rainfall data observed by AWS and estimated by QPM and IDW for overlapped locations in 2013 and 2014.

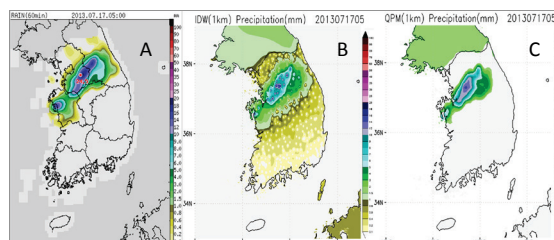


Figure 2. Comparison among maps of rainfall that were observed by AWS (A) and estimated by IDW (B) and QPM (C) at the spatial resolution of 1km × 1km in 2013.

Table 2. Contingency table of rice blast forecasts based on hourly rainfall data estimated by QPM and IDW as compared with the forecasts based on observed rainfall data for 49 overlapped locations in 2014.

Index	QPM	IDW
Hit	4801	3831
Miss	9200	10120
False alarm	9545	5199
Correct negative	42310	46656
Bias score	1.02	0.64
POD	34.3%	27.7%
FAR	66.5%	57.2%

DISCUSSION

QPM showed higher accuracy than IDW in estimating rainfall events and the amounts of rainfall (Table 1, Figs. 1 & 2). However, improvement in rice blast forecast was not certain when QPM data were used instead of IDW data. QPM provided higher POD in rice blast forecast than IDW, whereas it was not true for FAR (Table 2). The assumption of the Yoshino model regarding spore wash from plant surface due to hourly rainfall of ≥4mm caused increase in FAR when QPM data were used to forecast infection periods of rice blast.

POSTER 30

The crown rot deadhead phenomenon in durum wheat

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INTRODUCTION

Crown rot of wheat, caused by *Fusarium pseudograminearum* (*Fp*), is a serious disease threat across the Australian wheat belt, particularly in durum wheat. Control of this disease is primarily based on crop rotations and reducing inoculum, with a continued goal of producing crops with increased resistance. Plant reactions to disease are typically described using stem browning, sometimes coinciding with the production of deadheads, or stems undergoing premature senescence due to infection.

The mechanism by which crown rot causes yield loss has not yet been clearly described, however evidence is emerging indicating fungal interactions with both xylem and phloem tissues may play a role (1).

We hypothesise that stems exhibiting the deadhead symptom have more *Fp* biomass and greater vascular tissue colonisation than green living stems, resulting in their premature death. In less extreme infections this effect of stem colonisation may explain the physiological effects behind crown rot associated yield loss.

In order to test this hypothesis the levels of colonisation in stems of durum plants exhibiting deadheads were compared to stems of the same plants which were still living.

MATERIALS AND METHODS

Plant Materials Durum variety EGA Bellaroi was grown in *Fp* inoculated fields at Wellcamp, Qld. Plants were selected based on deadhead expression, where a single plant was exhibiting a deadhead and a living stem for comparison. Three data sets were collected across 3 years using approximately 30 plants each.

Stem Sections Three 6 cm sections were taken from the base of each stem, and the 6 cm of the peduncle below the head was also collected. Visual rating was performed on each section and DNA was then extracted for quantitative PCR analysis (2). Visual discolouration and *Fp* biomass was compared between sections from deadhead stems and living stems.

RESULTS AND DISCUSSION

In general, visual symptoms and the amount of *Fp* biomass decrease from the basal 0-6 cm section to the 12-18 cm section. Initial results indicate that deadhead stems typically have more severe visual symptoms than the infected living stems, however *Fp* biomass is not necessarily higher in deadhead stems (Figure 1).

The decrease in visual symptoms higher up the stem may reflect a diminishing layer of parenchymatous hypoderm. This is the layer which produces the brown discolouration used for visual assessment (1).

There was a wide range of colonisation levels, both between deadhead stems and living stems and within these groups. This is likely due to different stages of infection occurring simultaneously in the field, which cannot be distinguished using traditional visual methods.

Differences in infection levels between seasons are also highlighted in the *Fp* biomass measurements. These differences are not apparent in the visual rating data.

The top 6 cm of the peduncle has also had low, but detectable, levels of *Fp* in approximately 30% of stems.

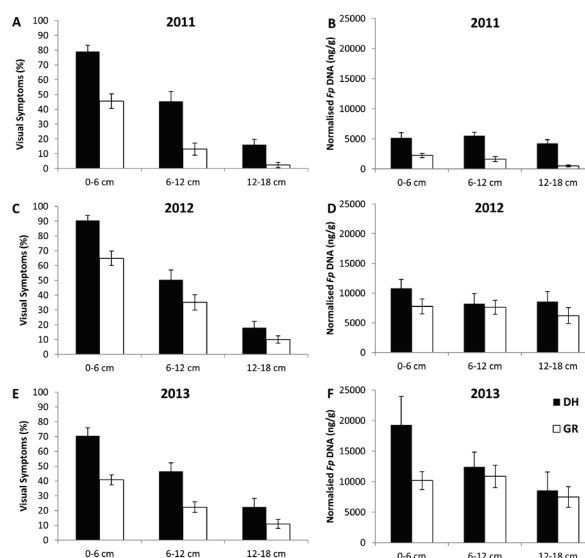


Figure 1. Visual symptoms (%) and *F. pseudograminearum* (*Fp*) biomass (ng/g) of deadhead (DH) and living (GR) stems of EGA Bellaroi in 2011 (A,B), 2012 (C,D) and 2013 (E,F). Three sections from 0-6 cm, 6-12 cm and 12-18 cm were collected from each stem. Bars represent the standard error.

Experiments are continuing on two further collections of durum plants from 2014, as well as several sets of bread wheat plants. This will include microscopic comparison of vascular bundle colonisation in sections from deadhead and living stems. The stem portion between 18 cm and the peduncle has also been retained for examination of fungal spread along a stem when *Fp* is detectable in the upper portion of the peduncle.

These experiments have indicated that deadhead formation is not simply a result of more prolific colonisation.

ACKNOWLEDGEMENTS

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POSTER 31

Common bean (*Phaseolus vulgaris*) seed lustre and country of origin can be indicators of resistance to *Pythium* hypocotyl diseaseYu Pin Li^A, Ming Pei You^A, Sally Norton^C and Martin J. Barbetti^{A,B}^A School of Plant Biology, The University of Western Australia, 35 Stirling Highway, Perth, WA, 6009, Australia^B The UWA Institute of Agriculture, The University of Western Australia, 35 Stirling Highway, Perth, WA, 6009, Australia^C Australian Grains Genebank, Department of Primary Industries Horsham Centre, 110 Natimuk Rd, Horsham, Victoria, 3400, Australia

INTRODUCTION

Common bean (*Phaseolus vulgaris*) is an important cultivated food legume, providing major dietary requirements for many cultures. There are high levels of variation in bean for many traits, including growth habit, seed characteristics, maturity and adaptation. The preferences for these and other characteristics differ from region to region, making breeding for traits such as disease resistance quite complex. Pre- and post-emergence damping-off is a major constraint to bean production worldwide, reducing both yield and quality (1). Of particular importance is damping-off caused by *Pythium* spp. This pathogen can cause yield losses of up to 70%. In parts of Australia, *Pythium* hypocotyl disease is the most widespread disease on common bean and *P. irregulare* is the most common species on common bean in Western Australia (2). Host resistance offers the most effective long-term disease management strategy for the control of *Pythium* root disease of common bean. Identifying effective host resistance could be enhanced if varieties with this characteristic could be identified through visible seed traits. Diverse varieties of common bean from 37 countries were screened for resistance against *P. irregulare* to determine relationships between *Pythium* hypocotyl disease resistance with country of origin and/or with seed lustre.

MATERIALS AND METHODS

A total of 194 bean varieties were challenged with *P. irregulare* isolate WAC4953. *P. irregulare* was cultured on potato dextrose agar (PDA) at 25°C in the dark until mycelium had almost grown across the plate. PDA plugs (1 cm x 1 cm) from the leading edge of a colony were cut and mixed with sterilised millet seeds to produce inoculum. The inoculum was incubated at 25°C in the dark for two weeks, taking care to ensure that the pathogen was evenly distributed among the seeds. Bean seed was sown 5 cm depth in free-draining pots (two seeds per 6 cm x 6 cm pot) filled with pasteurised potting mix containing. Each variety was grown in uninoculated soil and in soil containing 0.5% (w/w) colonised millet seed. Plants were grown at 18°C with a 12 h photoperiod. Each treatment was replicated four times. After 5 weeks, seedlings were scored for hypocotyl disease. Results for hypocotyl disease levels were compared with seed characteristics and country of origin data for test varieties

RESULTS

Bean varieties from Brazil and China had significantly less severe hypocotyl disease caused by *P. irregulare* than varieties from Japan and Italy (Fig. 1). Varieties with seeds that had either dull or intermediate lustre had significantly less severe hypocotyl disease than varieties with shiny lustre seeds (Fig. 2).

DISCUSSION

Host resistance offers the most effective and long-term disease management strategy for farmers to control common bean root disease. Utilising seed traits as a first

screen will facilitate the breeding and selection processes towards development and deployment of new common bean varieties with improved resistance to *Pythium* hypocotyl disease.

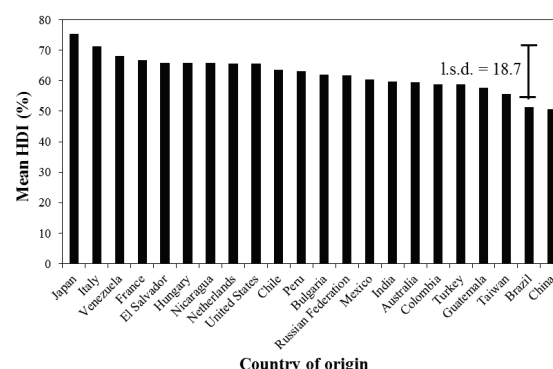


Fig. 1. Relationship between country of origin and *Pythium* hypocotyl disease index (HDI). A total of 194 common bean (*Phaseolus vulgaris*) varieties were screened, but 15 were excluded from the statistical analysis because they were the only variety from a particular country. The remaining 179 varieties were grouped by country of origin and ranked according to mean HDI. L.S.D = least significant difference at $P < 0.05$.

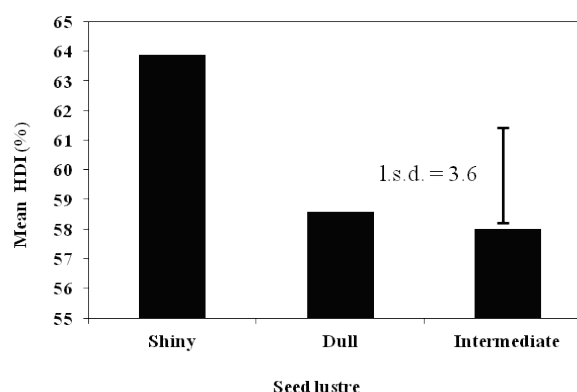


Fig. 2. Relationship between seed lustre and *Pythium* hypocotyl disease index (HDI). The 194 common bean (*Phaseolus vulgaris*) varieties were grouped by seed lustre and ranked according to mean HDI. L.S.D = least significant difference at $P < 0.05$.

ACKNOWLEDGEMENTS

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POSTER 32

Outbreak of *Cucumber green mottle mosaic virus* (CGMMV) in the Northern Territory, AustraliaL.T.T. Tran-Nguyen^A, D.A. Lovelock^A, M.Y. Voutsinos^A, S.J. West^B and J.R. Liberato^A^APlant Industries Division and ^BBiosecurity and Animal Welfare Division, Department of Primary Industry and Fisheries (DPIF), Australia

INTRODUCTION

Cucumber green mottle mosaic virus (CGMMV; *Tobamovirus*) occurs in 44 countries worldwide (1). Several strains have been identified and are known to mainly affect *Cucurbitaceae*. A typical infection will lead to a reduction in plant vigour, yield and fruit quality, ultimately affecting both farmer and consumer. CGMMV can be transmitted by infected seeds, plant to plant and mechanical inoculation.

In the Northern Territory (NT), Asian cucurbit vegetables are grown on ~90 premises and broad acre watermelon, melon and pumpkin crops are grown on ~15 premises. They are valued at ~AUD\$50 million annually, with the majority sold into southern Australia markets. In September 2014, CGMMV was detected for the first time in Australia, by a NSW plant pathologist, in a watermelon leaf sample from a survey carried out in July 2014 in Katherine NT. This initiated a biosecurity emergency response, led by NT DPIF, to delimit, contain and attempt to eradicate CGMMV.

MATERIALS AND METHODS

Surveillance: A surveillance program of cucurbit crops and associated weeds was undertaken, encompassing all cucurbit-growing regions of the NT. Commercial cucurbit farms in the NT have been surveyed to determine their CGMMV status and are continuously screened to support continuous trade movements.

Pathogen identification: Total RNA was extracted from plant leaf material using the Isolate II RNA plant kit (Bioline, Australia). Extractions were conducted in a Biohazard Class II cabinet within the Quarantine containment level 3 laboratory. Each RNA sample was subjected to PCR assays targeting the CGMMV coat protein and movement protein genes (2). Samples were also subjected to the CGMMV real time TaqMan RT-PCR assay (3). PCR products were purified using the QIAquick PCR purification kit (Qiagen, Australia). Sequencing was performed at the Australian Genome Research Facility and bioinformatics was conducted using the Geneious software.

RESULTS AND DISCUSSION

Pathogen molecular characterisation: The NT CGMMV strains showed very high sequence similarity (99.5 – 100%) to the CGMMV isolate from Indian bottle gourd (GenBank Accession number DQ767631) and the Canadian cucumber isolate (GenBank Accession number KP772568).

Eradication: On 24 March 2015, the National Management Group for CGMMV agreed that it was not technically feasible to eradicate the virus. By that time, CGMMV had been detected in 21 properties, in Darwin, Katherine and Ti Tree regions, on six commercial cucurbit species, four weeds genera, bees, pollen and bee hives.

Management program: The CGMMV response was changed to a management program on 25th March 2015.

All cucurbit crops intended for southern Australia markets are tested for CGMMV and issued a property freedom CGMMV phytosanitary certificate under the NT *Plant Health*

Act. Sampling includes 62 plants per individual planting for each plant host.

Surveillance: By the end of April 2015, 1320 and 479 single and composite samples (sampled from 10 or 11 plants), respectively, had been collected from 87 premises, the majority being cucurbit farms. CGMMV has been detected on 90 plant samples from 24 premises; on nine cucurbit crops namely *Benincasa hispida*, *Citrullus lanatus*, *Cucumis melo*, *C. sativus*, *Cucurbita maxima*, *C. moschata*, *C. pepo*, *Luffa* sp. and *Momordica charantia*; and on four weeds belonging to the genera *Amaranthus*, *Cucumis*, *Portulaca* and *Solanum*.

Compulsory seed tests: From 29th April 2015, the Commonwealth Department of Agriculture has made it compulsory that seeds from six cucurbit species imported into Australia must be tested for CGMMV and are free of the virus (Public Quarantine Alert PQA0989).

ACKNOWLEDGEMENTS

The authors acknowledge several tens of staff, including from interstate government departments, who have worked on the biosecurity response and now on the management program.

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POSTER 33

Postharvest diseases of mango in Fiji

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INTRODUCTION

Mango was introduced into Fiji in the late 1800s by early settlers (Cooper 1879 and Horne 1881, as cited in Iqbal 1982) (1). Despite this long history, mangoes in Fiji have been, and continue to be, produced predominantly on a semi-subsistence basis, characterised by fragmented plantings on small (<5 ha) orchards, growing primarily local varieties.

In contrast to the extensive literature published worldwide, there is very limited information available on postharvest diseases of mangoes in Fiji. Although improved cultivars have been imported, evaluated and released, little research has been carried out to explore the diseases which affect fruit of these cultivars after harvest.

MATERIALS AND METHODS

This paper reviews existing Pacific literature relating to current knowledge of postharvest diseases of mango in Fiji, and outlines new studies currently being undertaken to broaden this knowledge.

RESULTS AND DISCUSSION

Anthraxnose was first documented as a disease of mango in Fiji in 1956 (2), and its importance as a postharvest disease of mango noted in 1971 (1). Anthracnose not only makes the fruit unattractive, but also reduces fruit quality and subsequent market value. It was not until 1988 that the first report of mango anthracnose control in Fiji was published. The authors of this study investigated the efficacy of different methods of postharvest treatment (hot fungicide dips), which were applied to three varieties (Kensington, Haden and White Pirie) of mango grown in Fiji (3). Unfortunately they were unable to provide any definitive recommendations for postharvest treatments based on this work (3).

Anthraxnose is the only postharvest disease that has been formally reported to be present on mango in Fiji, with *Colletotrichum* sp. (teleomorph *Glomerella cingulata*) listed as its causal agent (Table 1). Other postharvest diseases would almost certainly occur on mango in Fiji, but to date have not been surveyed or reported. In the Secretariat of the Pacific Community (SPC) Pest List Database, the only other pathogens likely to cause postharvest disease of fruit, apart from *Colletotrichum* sp., are *Guignardia mangiferae* and *Cercospora mangiferae*, which are possible causes of dendritic spot, a sporadic problem in Australia (4).

Table 1. List of mango diseases present in Fiji according to the SPC Pest list Database (5)

Pathogen recorded	Common name of disease
<i>Cephaleuros virescens</i>	Algal spot
<i>Cercospora mangiferae</i>	Leaf spot
<i>Phellinus dingleyae</i>	
<i>Phanerochaete salmonicolor</i>	Pink disease
<i>Corticium salmonicolor</i> (synonym)	
<i>Gloeosporium</i> sp.	
<i>Glomerella cingulata</i> (teleomorph)	Anthraxnose
<i>Colletotrichum</i> sp.	
<i>Phyllosticta anacardiacearum</i>	
<i>Guignardia mangiferae</i> (teleomorph)	
<i>Kretzschmaria deusta</i>	
<i>Meliola mangiferae</i>	Black mildew

There is no listing of other mango postharvest pathogens commonly found throughout the world where mango is grown, such as the stem-end rot fungi (*Botryosphaeria* spp. and others). Therefore, there is a need to confirm the presence of these other postharvest diseases, not only for export purposes but for the improvement of the local domestic market, especially in regard to the local hotel industry which accounts for about 90% of total production. Given our limited understanding of current mango postharvest diseases in Fiji, our research will include a survey to determine the presence of these diseases, identification of the causal agents, and an assessment of disease management options. A more detailed characterisation of *Colletotrichum* species causing mango anthracnose in Fiji, based on current molecular phylogenetics, will also be undertaken as part of this study.

ACKNOWLEDGEMENTS

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POSTER 34

Efficacy of selected bactericides in reducing fruit lesions caused by *Pseudomonas syringae* PV. *syringae*

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INTRODUCTION

Bacterial canker, caused by *Pseudomonas syringae* pv. *syringae* van Hall (Pss) and *Pseudomonas syringae* pv. *morsprunorum* (Psm), is an important disease of stonefruit worldwide. Options for control of bacterial canker are limited and heavily dependent on copper products and streptomycin. However, the efficacy of copper is limited by its protectant mode of action, the potential for phytotoxicity and the emergence of copper resistant strains. In a recent *in vitro* study, 80% of the Pss strains collected from 8 sites in Central Otago, New Zealand, were found to be copper-resistant[1]. The objective of this work was to test different commercial formulations of bactericides, including one copper-based products to determine their efficacy in reducing lesions caused by Pss using plant systems.

MATERIALS AND METHODS

Copper (as Kocide Opti), kasugamycin (as Kasumin) and a novel bactericide (CSA2014) were tested for their efficacy in preventing necrotic lesions on detached fruits of ‘Rainier’ cherry. Immature cherry fruits were surface sterilised by immersion on 1% sodium hypochlorite, washed with distilled water, and dried in a laminar flow cabinet. Each fruit was wounded three times (2mm depth) with a sterile toothpick and treated with 10 µL of each concentration of each product (Table 1). After drying at room temperature for 1 h, 10 µL of a bacterial suspension (10⁸ CFU/ml) of a copper resistant strain of Pss (PFR Vlr1.1) was inoculated onto the treated wounds. Controls included sterile water (W), Pss only and chemical only treatments. Fruit were arranged in trays, with 3 rows. Each tray contained one fruit treated with water only, and one just inoculated with Pss only. The remaining fruit in the tray consisted of one fruit treated with each concentration of one particular bactericide with and without Pss inoculation (one fruit per combination). There were six trays per chemical. Trays were incubated at 22°C for 7 days. After incubation, the percentage of wounds showing necrosis and the lesion size were recorded. The %wounds showing necrosis were analysed with a binomial generalised linear model with a logit link [2]. The log(Lesion size) for wounds that were necrotic (sizes >0 only) were analysed with a mixed model fitted with restricted maximum likelihood [3], with trays and individual fruit as random effects and treatments as a fixed effects.

RESULTS AND DISCUSSION

No unionoculated fruit developed lesions. Results for inoculated fruit are summarised in Figure 1. All wounds inoculated with Pss only developed necrotic lesions. For inoculated wounds treated with any of the bactericides, the necrosis incidence declined with increased concentrations with no necrosis at the highest rates. Overall Kasumin showed the greatest efficacy in preventing necrosis at the recommended concentration. CSA2014 and Kocide Opti at the recommended rate failed to prevent necrosis but did reduce lesion size. All bactericides were able to provide protection against the copper resistant strain of Pss. These

results suggest that copper products may still be exerting some control of Pss despite the high incidence of copper tolerant strains in the population.

Table 1. Concentration of each bactericide used. Numbers in bold are recommended commercial concentrations.

Treatment code	CSA2014 mg/L	Kocide Opti g/L	Kasumin mL/L
1	25	0.78	1.25
2	50	1.56	2.5
3	100	3.12	5
4	150	4.68	
5	200	6.24	

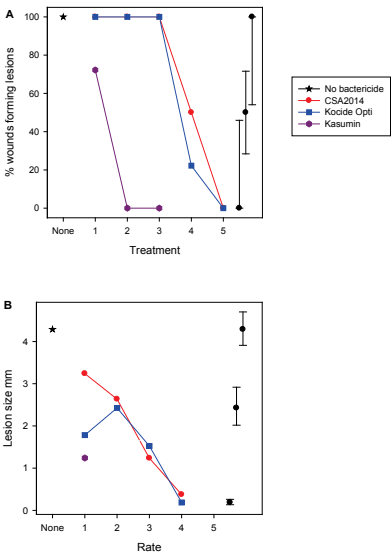


Figure 1. Pss inoculated fruit: A) Percentage of wounds developing necrotic lesions and B) Mean lesion size for wounds that formed necrotic lesions on detached ‘Rainier’ cherry fruit in relation to bactericide, concentration. Errors bars are for a small, large and mid-range mean.

ACKNOWLEDGEMENTS

Funding for this study was provided by Sumerfruit New Zealand and PFR. Thanks to Etec Crop Solutions and DuPont for supplying bactericides.

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POSTER 35

Sensitivities of New Zealand isolates of *Zymoseptoria tritici* to succinate-dehydrogenase-inhibiting (SDHI) fungicides

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INTRODUCTION

Speckled leaf blotch (aka *Septoria tritici* blotch), caused by the fungal pathogen *Zymoseptoria tritici*, is a serious disease of wheat in New Zealand and worldwide. Yield losses are severe when disease affects the final three leaves of plants. Disease outbreaks in recent years in New Zealand have started earlier in the season and have been more severe than previously observed. Our recent studies have shown that populations of *Z. tritici* are no longer controlled by the quinone outside inhibitor (QoI) fungicides (unpublished data). Farmers have now to rely on other groups of fungicides, such as the demethylation inhibitors (DMIs) and the more recent succinate-dehydrogenase-inhibiting (SDHI) fungicides to control the disease. Because of their single-site mode of action, SDHI fungicides are considered at medium to high risk of resistance development by *Z. tritici*. In a fungicide resistance management strategy, it is important to know the baseline level of fungicide sensitivity, before the development of resistance. Our objective was to determine the sensitivity of Canterbury isolates of *Z. tritici* to the newly introduced SDHI fungicides.

MATERIALS AND METHODS

Leaves showing symptoms of *Z. tritici* blotch were collected from 17 commercial wheat paddocks in the Canterbury region during the 2014-15 growing season, and single pycnidium isolates were obtained. The sensitivity of selected isolates to technical grade izopyrazam, bixafen and fluxapyroxad was determined using a microplate testing method [1] with eight concentrations for each fungicide. The New Zealand reference sensitive strain 2SC2, isolated in 2003 prior to the introduction of SDHIs in New Zealand, was included in all tests. Microplates were incubated at 23°C for 4 days on an orbital shaker at 150 rpm. Growth was determined by optical density using a Molecular Devices ThermoMax microplate reader at 450 nm. The half maximal effective concentration (EC₅₀ in µg/mL) was determined using a dose-response relationship according to the analysis software SoftMax® Pro 5.4.1.

RESULTS AND DISCUSSION

The EC₅₀ values for a total of 340, 335 and 323 single pycnidium *Z. tritici* isolates were calculated for isopyrazam, bixafen and fluxapyroxad respectively. For isopyrazam, the EC₅₀ values varied from 0.001 to 0.095 mg/L, with an average value of 0.021 mg/L. For bixafen, the EC₅₀ values varied from 0.001 to 0.096 mg/L, with an average value of 0.034 mg/L. The EC₅₀ values for fluxapyroxad were in the range of 0.001 to 0.096 mg/L, with an average value of 0.039 mg/L. The EC₅₀ values for the reference strain 2SC2 were 0.001, 0.010 and 0.003 for isopyrazam, bixafen and fluxapyroxad, respectively.

All isolates were considered sensitive to the three SDHI fungicides tested. The higher average EC₅₀ values obtained for the tested isolates compared with the EC₅₀ value for the reference strain is likely to reflect the variation within the population rather than shifts in sensitivities.

The SDHI fungicide group is an important tool for control of *Septoria tritici* blotch. This work provides valuable information in relation to the range of sensitivity within the Canterbury population of *Z. tritici* at the very early stages of SDHI use in New Zealand. Future work will include further monitoring of *Z. tritici* populations, contributing to a resistance management strategy.

ACKNOWLEDGEMENTS

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POSTER 36

Outbreak of freckle disease on Cavendish bananas in the Northern Territory, Australia

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INTRODUCTION

Freckle disease of bananas is an economically important disease in parts of Asia and the Pacific Islands. Freckle disease is associated with three species of *Phyllosticta*, including *P. maculata*, *P. musarum* and *P. cavendishii*. Only the latter is known to infect Cavendish bananas (1). Symptoms of the disease are raised black spots on leaves and fruits. Freckle disease reduces yields and the marketability of blemished fruit, leading to increased fungicide use and production costs associated with controlling the disease.

The Australian banana industry is worth \$600 million annually and Cavendish bananas account for 95% of the market. The strain of *P. cavendishii* affecting Cavendish (previously referred to as *Guignardia musae*) is a pest threat priority in the banana industry biosecurity plan and a Category 3 Emergency Plant Pest under the Emergency Plant Pest Response Deed (EPPRD).

Phyllosticta cavendishii has been present in the Northern Territory (NT) since at least 1991 on Lady Finger and other non-Cavendish bananas. However, the disease was not detected on Cavendish bananas prior to 2013, despite being targeted in routine surveys conducted by Northern Australia Quarantine Strategy plant pathologists since 2002.

In July 2013, *P. cavendishii* was detected on Cavendish bananas at a residential property near Darwin in the NT. In response to this disease outbreak, the National Banana Freckle Eradication Program (NBFEP) was established, led by NT DPIF and approved by the National Management Group under the EPPRD.

MATERIALS AND METHODS

Surveillance A large-scale surveillance program of urban, semi-urban and rural premises was undertaken mainly in the Darwin-Daly weather district. Commercial banana farms were surveyed periodically.

Pathogen identification Samples were carefully examined under a microscope for disease symptoms (i.e. *Phyllosticta* pycnidia). Total DNA was extracted from infected banana leaf using a commercial kit (Qiagen, Australia). Two PCR tests were conducted: (i) ITS PCR using primers GmF/GmR2 that produces a band of 430 bp (1) and (ii) Phrm PCR using primers PhrmF/PhrmR that produces a band of 208 bp (2). The ITS PCR product was purified and then sequenced using the BigDye Terminator mix at the Australian Genome Research Facility. Sequences were analysed using the Geneious Bioinformatics platform. BlastN from NCBI was used to initially identify the *Phyllosticta* species. Additional phylogeny studies were conducted to construct maximum likelihood phylogenetic trees using reference *Phyllosticta* type strains.

Banana cultivar identification Plant DNA from all samples with freckle disease was extracted and analysed using PCR and DNA fingerprinting to identify cultivar (3, 4).

RESULTS AND DISCUSSION

Surveillance By the end of October 2014, 32,296 premises, mainly residential, had been surveyed. Of those premises, 8,258 had banana plants and were surveyed for freckle disease. A total of 1,395 samples were collected for disease diagnoses from 676 premises.

Diagnostics Freckle disease was detected on 465 samples from 268 separate premises (Table 1). *P. cavendishii* was detected in Darwin city and Greater Darwin Rural Area, Batchelor, Naiyu, Ramininging, Dundee Beach and Melville Island.

Eradication Initially the strategy was to eradicate all bananas plants within a 1 km radius of a premise with freckle-infected Cavendish bananas. From October 2014, it was decided that all banana plants within six infestation zones were to be destroyed by the end of April 2015.

Table 1. Premises with freckle-infected banana plants in the NT (July 2013 - October 2014).

Cultivar	<i>P. cavendishii</i>	<i>P. maculata</i>
Bluggoe (ABB)	4	10
Cavendish (AAA)	61	-
Ducasse (ABB)	9	-
Lady Finger (AAB)	183	4
Silk (AAB)	42	-
Others	3	-
Total ¹	256	13

¹Multiple infected cultivars occurred in some premises. One premise had both *P. cavendishii* and *P. maculata*.

ACKNOWLEDGEMENTS

We acknowledge all of the NT and interstate/Commonwealth government department staff who worked on the NBFEP until Oct 2014 (<http://www.nt.gov.au/d/Content/File/p/AR/2013-14-DPIF/AR-201314-DPIF.pdf> - p.70-72).

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POSTER 37

Molecular and pathogenic structure of the current Australian *Ascochyta rabiei* population

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INTRODUCTION

Blight caused by *Ascochyta rabiei* is a devastating disease of chickpea in Australia. The quality of the seed becomes unmarketable and complete yield losses have been reported [1]. Knowledge of variation and, monitoring for, changes in the isolate population (molecular and pathogenic) within and among chickpea growing regions is required to assess the risk for the pathogen to adapt to potentially overcome disease management strategies. In particular, surveillance will help to determine if the pathogen is changing over time and in association with widely deployed resistant cultivars. This will also enable the identification of the highest risk isolates for selective resistance breeding purposes.

MATERIALS AND METHODS

For a comparative study with previous annual populations, 158 *A. rabiei* isolates were hierarchically collected from within the major Australian chickpea growing regions and from commonly grown cultivars in 2013. Isolates were genotyped with the 10 most informative SSR loci previously identified via fluorescent PCR and capillary electrophoresis [2].

The pathogenicity of each 2013 isolate was determined under controlled bioassay conditions [3] on three key resistant cultivars/accessions (PBAHatTrick, Genesis090 and ICC3996) and a susceptible check (Kyabra).

RESULTS

The five most useful loci varied in their ability to explain the relationship among the isolates from 0.156 (ArH05T) to 0.016 (ME-14-1-83) (Table 1). These were subsequently used to identify haplotypes, haplotype frequencies and calculate the 2013 population diversity measures. Many of the haplotypes previously detected [2] were detected together with 18 new haplotypes (ARH125-ARH142). The most frequently detected haplotype was again ARH01, which accounted for 68% of the isolates collected and included isolates with high pathogenicity on Genesis090 and PBAHatTrick. The total population diversity detected across Australia in 2013 was 0.057, which is very low compared to other global regions and similar to that detected previously within Australia [4]. This indicates that as a whole, the population has not significantly increased in gene diversity recently, a likely factor of clonal propagation and lack of sexual recombination or major new introductions. Interestingly, significantly more diversity was detected among isolates found on the resistant cv. PBASlasher than on other commonly grown cultivars.

The pathogenicity of each 2013 isolate was scrutinised and several pathogenicity groups were detected. Group 1 caused severe disease on PBAHatTrick but not on other resistance sources; Group 2 caused severe disease on PBAHatTrick, moderate disease on Genesis090 and low disease on ICC3996; Group 3 caused severe disease on PBAHatTrick, moderate disease on Genesis090 and moderate disease on ICC3996; Group 4 caused severe disease on PBAHatTrick, high disease on Genesis090 and

moderate disease on ICC3996. Note that sporulation was not detected for any isolate on ICC3996. All Group 3 and 4 isolates were collected from Kingsford in South Australia, from the same plant sample of Genesis090. Several of the northern NSW isolates were highly pathogenic on PBAHatTrick but unable to cause significant disease on Genesis090. Some isolates from PBASlasher were highly pathogenic on Genesis090. None of the QLD isolates caused significant disease on any of the resistant sources assessed.

Table 1. List of five polymorphic loci

Locus	Size of Allele	Diversity
ArA03T	412, 415, 430, 409	0.116
Arh05T	239, 224, 242, 248, 233	0.156
Mel-14-1-56	379, 383	0.016
Mel-14-1-63	316, 313	0.035
Mel14-1-83	283, 285	0.016

DISCUSSION

Whilst the overall molecular structure of the population appears to be static, we have detected isolates from the most recent population able to cause significant disease on our most resistant cultivars. This indicates that there is sufficient variation within the current mainly clonal population to enable selection of isolates and potential for population adaption to overcome resistance genes used within the National Breeding Program.

Isolates of a large haplotype group (highly clonal) and pathogenic on the best currently deployed resistance sources were deemed to be of the highest risk to the chickpea industry and were supplied to the national breeding program for screening of advanced breeding material and existing cultivars.

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POSTER 38

Seasonality of *Phyllosticta* spp. in citrus leaf litter

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INTRODUCTION

Citrus in most tropical and subtropical regions are host to *Phyllosticta* spp., the most important of which is the cause of citrus black spot (CBS), *P. citricarpa*. Also present is the morphologically identical endophyte *P. capitalensis* (1). The lifecycle of both fungi includes a significant phase within leaf litter, however little is known about the seasonal dynamics of the two species during this time.

Both species are reported to form pycnidia, pseudothecia and spermogonia, each producing conidia, ascospores and spermatia, respectively. Ascospore production is considered the most important factor in CBS epidemics, but previous studies of seasonal dynamics have been hampered by the morphological similarity of the two species.

In order to better understand the seasonal dynamics of the two species in citrus orchards in Queensland (Qld), leaf litter sampling, spore harvesting and species-specific qPCR will be used to determine the relative contributions of each species to each spore type. This information will then be used to improve disease control strategies in commercial orchards.

MATERIALS AND METHODS

Leaf collection During the first 20-24 weeks of fruit development, when fruit are susceptible to CBS, 600 leaves were sampled each fortnight from the leaf litter of three commercial orchards in Qld. Each leaf was inspected under a dissecting microscope for the presence of fructifications typical of *Phyllosticta*. Leaves without fructifications were discarded. The portions of leaves with fructifications were excised, and the proportions of spermogonia, pycnidia, and pseudothecia present on the leaves was determined by light microscopy.

Spore extraction. The various spore types were extracted from the leaf tissue by stimulating aerial ejection of ascospores, followed by liberation of spermatia and conidia into water. The spores were quantified then frozen for storage.

Quantitative PCR. DNA was extracted from leaf tissue and spore suspensions, and qPCR performed using existing methods (2).

RESULTS

Results have so far demonstrated that spermogonia and pycnidia are generally present throughout the susceptible period, with the latter peaking in the earlier half (Fig. 1). In the second half of the susceptible period pseudothecia production increased significantly, indicating a shift from clonal to sexual reproduction. qPCR results are pending, but early results suggest *P. citricarpa* to be the dominant species in Qld orchards.

DISCUSSION

Our findings suggest that infection of fruit via airborne ascospores is most likely to occur in the second half of the

susceptible period. The timing of pseudothecia production indicates that mating events may occur in the first half of the susceptible period. Preliminary qPCR results showing *P. citricarpa* to be the dominant species is likely to be the result of CBS being present in Qld for at least 100 years. Further results from these experiments will test these various hypotheses.

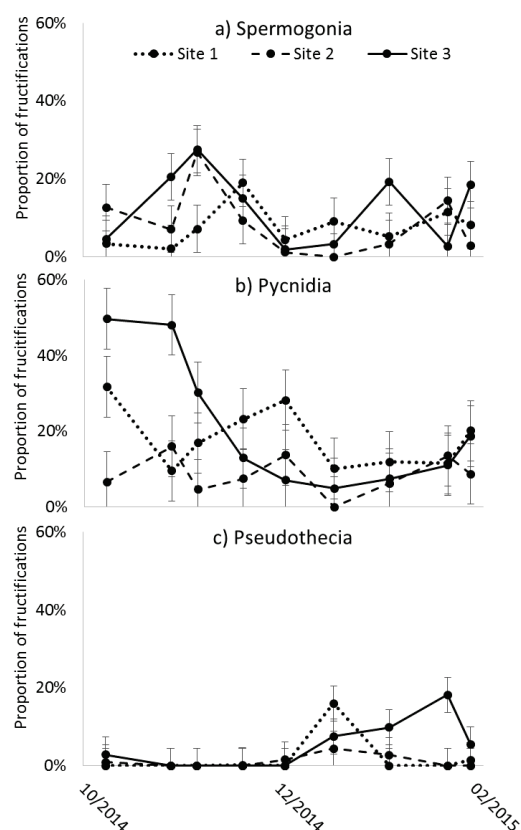


Figure 1. Proportions of a) spermogonia, b) pycnidia, and c) pseudothecia of *Phyllosticta* spp. present on *Citrus* leaves sampled from the leaf litter in three commercial orchards in Qld during the period of fruit susceptibility. Bars represent least significant difference.

ACKNOWLEDGEMENTS

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POSTER 39

Analysis of the microflora of an Australian native psyllid that feeds on solanaceous species

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INTRODUCTION

Zebra chip disease of potato, caused by the gram negative bacteria *Candidatus Liberibacter solanacearum* (Lso)(1) poses a severe threat to Australia's potato industry. The tomato/potato psyllid, *Bactericera cockerelli* is able to vector Lso, transmitting the bacteria while feeding on solanaceous crops such as potatoes, tomatoes, eggplants and peppers. However, this psyllid species has not been reported in Australia. The incursion of zebra chip disease in New Zealand cost potato growers an estimated \$200 million and the vector has recently been detected in Norfolk Island (1,2). Australia is a centre of psyllid diversity with hundreds of native species present. Among these are native psyllids that have been reported to feed on host species that are susceptible to pathogenic *Liberibacter*. For example *Acizzia solanicola* is known to feed on eggplants and other solanaceous plant species spread throughout Australia. It is unknown if this psyllid species can vector Lso. Native Australian psyllids may also harbour microflora that are closely related to pathogens such as *Candidatus Liberibacter* species, which can potentially confound the diagnostic protocols for exotic Emergency Plant Pests (EPPs) surveillance. To investigate the potential role of Australian psyllids in transmitting *Liberibacter* species, we have developed general primers to test for all *Liberibacter* species using conventional polymerase chain reaction (PCR) test and adopted a next generation sequencing approach to profile the microflora of Australian native psyllids.

MATERIALS AND METHODS

General *Liberibacter* primers based around the 16S region were designed to amplify sequences from across the *Liberibacter* genus encompassing both pathogenic and non-pathogenic strains while excluding closely related bacteria. The primers were validated using three species of *Liberibacter*; *Liberibacter asiaticus* (Las), *Liberibacter europeas* (Leu) and Lso. These primers were used to screen DNA extracts from native Australian psyllid, *Acizzia solanicola*, collected from solanaceous plants from Victoria and New South Wales in Australia to identify any *Liberibacter* species associated with these insects. Next generation sequencing was used to further investigate the microflora associated with these psyllids and to gain an understanding of the microflora profile within an Australian native psyllid.

RESULTS AND DISCUSSION

The general *Liberibacter* primers developed were able to amplify DNA extracted from individual insects indicating a *Liberibacter* species is present in *Acizzia solanicola*. The primers were successfully validated for

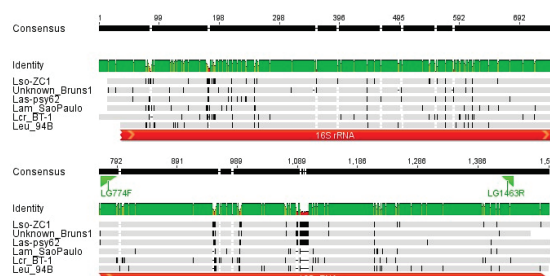


Figure 1. Comparison of the 16S rRNA region of Lso (NR_074494.1), an unknown bacteria present *A. solanicola* (Unknown_Bruns1), Las (NR_074528.1), *Liberibacter americanus* (Lam)(FJ914621.1), *Liberibacter cresens* (Lcr) (NR_102476.1) and Leu (JX244260.1). The forward (LG774F) and reverse (LG1463R) general *Liberibacter* primers are represented by the green triangles against the Consensus sequence. Geneious version 7.1 created by Biomatters. Available from <http://www.geneious.com>

Las, Leu and Lso. The full 16S region, approximately 1500 bp of the *Liberibacter* species was sequenced from *Acizzia solanicola* that gave a positive PCR result and BLAST analysis confirmed the presence of a *Liberibacter* species with 99% identity.

Most *Liberibacter* species are currently unculturable (3). Therefore to analyse the microflora profile of the psyllid, total DNA extracts from psyllid samples were sequenced using next generation sequencing. *Liberibacter* species and other microflora are present in very low titre in the host psyllid, so deep metagenome sequencing was required to characterise these communities.

Increased knowledge of the microflora of psyllids, including the presence of *Liberibacter* species in Australian native psyllids will aid Plant Biosecurity diagnostics globally, to support development of accurate diagnostic tools. It is anticipated that the dominant endosymbionts that colonise *A. solanicola* will be identified and their role in microbial ecology of native psyllids will be presented and discussed.

ACKNOWLEDGEMENTS

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POSTER 40

Isolation and identification of *Fusarium* species associating with yield decline of pyrethrum in TasmaniaA. Moslemi^A, T. Groom^B, Peter Ades^A, Marc Nicholas^A, P.W.J. Taylor^A^AFaculty of Veterinary and Agricultural Science, University of Melbourne, VIC, 3010, Australia^BBotanical Resources of Australia, Burnie, TAS 7320, Australia; Email: a.moslemi@student.unimelb.edu.au

INTRODUCTION

Pyrethrum is a perennial plant cultivated commercially for the production of natural insecticides, from the oil glands inside the seeds. Pyrethrum yield decline syndrome is where patches of plants in pyrethrum paddocks were not able to regrow after first harvest [1] resulting in significant yield losses in Tasmania during the last decade. High yielding crops are often terminated prematurely after first harvest. Pyrethrum plants in yield decline affected fields showed less density in autumn and after the first harvest, most plants were not vigorous enough to regrow or had lower yield in the second harvesting season [2, 3]. Preliminary analysis of yield decline affected plants consistently isolated several *Fusarium* species. The aim of this study was to isolate and identify pathogenic *Fusarium* species associated with yield decline syndrome of pyrethrum plants in Tasmania.

MATERIAL AND METHODS

First sampling was in June 2014 from 9 yield decline affected sites and tissues from the root, crown, petiole and leaf were cultured on water agar (WA) and then hyphae was cultured onto potato dextrose agar (PDA) for isolate identification. A second sampling time was undertaken from three of the previously sampled sites in January 2015. Healthy plants from sites adjacent to the yield decline sites were also sampled. Molecular identification of *Fusarium* spp. was carried out using three most informative genes, individually and combined, ITS, RPB2 and *tef1* [4]. Phylogenetic trees were constructed and isolates were identified to species level. Two glasshouse trials were set up to assess pathogenicity of the *Fusarium* by either dipping the roots of young pyrethrum plants in 10⁴ spore/ml spore suspension then replanting into potting mix; or spraying the leaves of young plants in pots using a hand sprayer. Inoculated plants were maintained in the glasshouse for two months at which time disease symptoms were noted, then plants were sampled and tissue from the roots, crown and leaf petioles were cultured to isolate the *Fusarium* spp. Also the below and above ground biomass was measured by drying the plants in oven for three days at 71°C.

RESULTS AND DISCUSSION

Four different *Fusarium* species (based on morphotypes in culture); were isolated from the roots, crowns, petioles and in rare cases from leaves of plants from yield decline sites. No *Fusarium* spp. was isolated from healthy plants. Phylogenetic analysis identified the species as *F. oxysporum*, *F. chlamydosporum*, *F. venenatum* and *F. incarnatum-equiseti*. From the first sampling time, *F. oxysporum* and *F. chlamydosporum* were predominately isolated from 3 out of 9 sites; and mostly from the crown, root and petiole tissues. Isolation of *F. incarnatum-equiseti* occurred only in rare cases (one site) in the first sampling time. *Fusarium venenatum* was only isolated rarely from one site in the first sampling time and also one site but frequently in the second sampling along with *F. oxysporum* and *F. chlamydosporum* which were isolated from all

3 sites. In the second sampling > 90% of the plants had the crown tissue infected with *F. oxysporum*, *F. chlamydosporum* or *F. venenatum*; root and petiole tissue infected with *F. oxysporum* or *F. chlamydosporum*; and leaf tissue infection with *F. chlamydosporum* or *F. venenatum*. No *F. incarnatum-equiseti* was isolated in the second sampling time.

In the first glasshouse trial, although no obvious disease symptoms were observed, plants inoculated with *F. oxysporum* and *F. chlamydosporum* had reduced plant growth and were less vigorous. These species were readily isolated from the root, crown and petiole tissues of inoculated plants. There was no effect on plant growth of plants inoculated with *F. incarnatum-equiseti* and this *Fusarium* spp was not reisolated from inoculated plants. The second glasshouse trial with *F. oxysporum*, *F. chlamydosporum* and *F. venenatum* has yet to be harvested. Further sampling of yield decline affected sites will be necessary to provide evidence on the association of these *Fusarium* species with yield decline of pyrethrum.

ACKNOWLEDGMENTS

This project was supported by Botanical Resources Australia-Agricultural Service Pty. Ltd. The author gratefully acknowledges the growers who allowed access to their crops for sample collection.

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POSTER 42
Secondary metabolome of the forest pathogen *Dothistroma septosporum*

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INTRODUCTION

Dothistroma septosporum is a fungus causing the disease Dothistroma needle blight (DNB) on more than 80 pine species worldwide, and causing severe economic losses (1). According to a recent study, there are 11 core secondary metabolite (SM) genes in the *D. septosporum* genome, which is lower than found in closely related species (2). Recently the SM dothistromin was identified as a virulence factor (3). The aim of this project is to find out if SMs produced by any of the other ten predicted SM genes play an important role in DNB.

MATERIALS AND METHODS

Domain structure and metabolite product prediction. The sequences of the SM genes were gathered from the JGI database (4), and the domains identified using a combination of 'PKS/NRPS analysis website' (5), 'Domain search program for NRPS and PKS' (6) and manual identification of SAT domains (7). Determination of predicted pathway product types was performed via NAPDOS analysis tool (8, 9).

Transcriptome analysis. Transcriptome analysis was performed on epiphytic/biotrophic (early), initial necrosis (mid) and mature sporulating lesion (late) stages *D. septosporum* infections on *Pinus radiata* seedlings (10). RNA-seq data was used to find out whether the fungal SM genes were expressed *in planta*.

Phylogenetic analyses. Phylogeny of Pks2 has been studied based on best BlastP matches within the JGI database. Phylogenetic trees were built using PhyML with bootstrap of 1000.

RESULTS AND DISCUSSION

Gene	Domains	Predicted pathway product type
PksA	SAT-KS-AT-ACP-ACP-TE	Dothistromin ⁽²⁾
Pks1	SAT-KS-AT-ACP-ACP-TE	Melanin/Naphtopyrone
Pks2	KS-AT-DH-ER-KR-ACP	Fumonisin
Pks3	KS-AT-ER-KR	Epothilone
Pks4	Probably pseudogenised	
Nps1	C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C	Cyclosporin
Nps2	A-C-A-T-C-T-A-C-C	Siderophore/Bacitratin
Nps3	A-T-C-A-NM-T	Cyclosporin
Hps1	KS-AT-CM-KR-C-A-T	Cyclopiazonic acid
Hps2	KS-AT-CM-KR-C-A-T	Compactin/Tyrociclin
Dma1		

Table 1. *D. septosporum* SM genes and characteristics of their products. SAT: starter unit ACP transacylase, KS: keto-synthase, AT: Acyltransferase, ACP: Acyl carrier protein, TE: Thioesterase, DH: Dehydratase, ER: Enoylreductase, KR: Ketoreductase, C: Condensation, A: Adenylation, T: Thiolation, NM: N-methylation, CM: C-Methylation.

Domain structures and metabolite predictions were used in knockout preparations by targeting essential domains of the genes, and providing basis for future plans in biochemical profiling. Transcriptome analysis results showed that *Pks1*, *Pks2*, and *Nps3* have expression levels as high as the dothistromin gene *PksA*, although their patterns

of expression are different, with *Nps3* being most highly expressed at early stage (Figure 1).

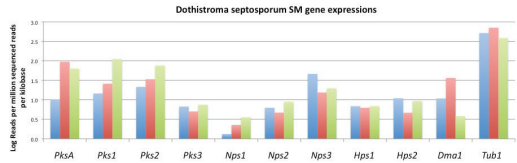


Figure 1. *D. septosporum* SM gene expression on *P. radiata* seedlings at early, mid, and late stages.

Proteins with high similarity to *D. septosporum* Pks2 are produced by diverse species such as the opportunistic human pathogen *Aspergillus terreus* and the root rot pathogen, *Zopfia rhizophila*. Preliminary phylogenetic analyses of Pks2 suggested possible horizontal gene transfer. However increasing the number of taxa showed *D. septosporum* Pks2 and the similar genes from closely related species are more likely paralogs.

CONCLUSIONS AND FUTURE WORK

Based on the expression data, Pks1, Pks2, and Nps3 are potential virulence factors. Knockout and functional analyses for these genes are in progress.

According to the phylogenetic analyses, there is no evidence that *Pks2* was gained by horizontal gene transfer.

Dothistromin deficient mutant $\Delta PksA$, mutants of global SM regulators $\Delta LaeA$ and ΔVeA , and wild type *D. septosporum* spores are being used to compare the SM productions under different conditions such as different nitrogen source, carbohydrate concentration and iron deprivation to induce production of some of the SMs. Differences in the SM profiles will be analysed and ultimately the metabolites extracted for biochemical analysis.

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POSTER 43

Azoxystrobin offers potential for chemical control of rice blast (*Magnaporthe oryzae*) in AustraliaDolar Pak^A, Ming Pei You^A, Vincent Lanoiselet^B, Martin J. Barbetti^A^ASchool of Plant Biology, University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia^BDepartment of Agriculture and Food Western Australia, Baron-Hay Court, South Perth, W.A. 6151, Australia

INTRODUCTION

Magnaporthe oryzae, anamorph *Pyricularia grisea*, is one of the most devastating pathogens of rice, causing rice blast disease that annually destroys rice yield sufficient to feed 60 million people (1). *M. oryzae* can infect rice plants at any stage of its growth from seedling to grain formation and causes nodal blast, collar rot, leaf blast, neck blast and/or panicle blast (5). In addition, it has been shown that *M. oryzae* can also infect roots of rice plants and systemically move from root to aerial tissues and still cause blast disease (4). Blast disease can be managed by various combinations of using resistant cultivars, by flooding rice fields, by avoiding excessive use of nitrogen, by changing planting dates, and by spraying fungicides. However, the fungicide option is preferred by farmers in controlling blast once symptoms are evident in the field (2). In Australia, rice blast disease has recently become a problem in paddy fields in Ord River Irrigation Area in Western Australia (6), but primary production areas including Murrumbidgee, Coleambally and Murray Valley irrigation areas in southern New South Wales (3) are still free from the disease. Currently, only two fungicides known for controlling blast disease outside Australia are registered within Australian Pesticides and Veterinary Medicines Authority (APVMA); but both available chemicals, Azoxystrobin and Propiconazole, are still not registered for use in controlling rice blast disease (APVMA database) in Australia. Therefore, the objective of these studies was to test the baseline sensitivity of Azoxystrobin in preventing *M. oryzae* conidial germination and germ tube elongation.

MATERIALS AND METHODS

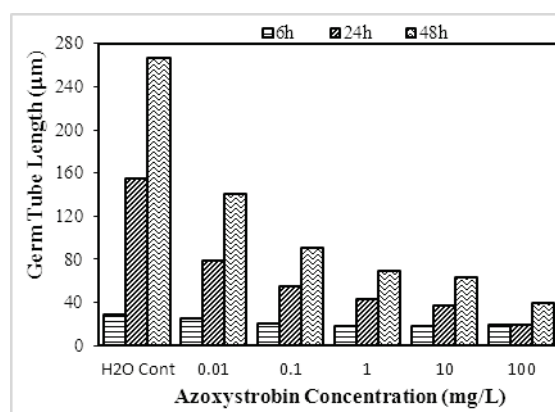
Chemical effect on *M. oryzae* conidial germination and germ tube elongation. For this study, *M. oryzae* WAC13466 was used and the test concentration was prepared from stock chemical solution (0.25% w/v). Cover slips placed on glass slides were used as the materials for inoculating *M. oryzae* conidia. The inoculum density and volume in this experiment was 2×10^5 conidia/ml and 30 μ l, respectively. All cover slips inoculated with conidia were immediately placed in a moist chamber and incubated at 23°C in the dark. Conidial germination was monitored at 6, 24, and 48 hr by light microscopy.

Fungicide spraying experiment. Susceptible rice cv. Quest was used in this experiment and the seedlings were grown until 4-weeks-old in glasshouse. Azoxystrobin at the concentration of 200, 100, 0.68 mg/L was sprayed on to seedlings, allowed to dry and seedlings subsequently inoculated with a conidial suspension (10^5 conidia/ml). The 200mg/L rate was based upon the recommended dose for powdery mildew in cucurbits; the half commercial concentration of Azoxystrobin and EC₅₀ (i.e., 0.68mg/L) were chosen as dilutions of the recommended dose for powdery mildew. Disease assessments were performed at 7 days after inoculation utilising a disease scoring scale of 0-5. The experiment was repeated twice.

RESULTS AND DISCUSSION

Results indicated that the Azoxystrobin was very effective against the rice blast isolate from Western Australia. The concentration needed to impede conidial germination and germ tube elongation was much lower compared with the recommended commercial dose in disease/crop combinations other than rice blast. The EC₅₀ calculated separately from a separate sensitivity test on mycelium growth was 0.68mg/L. The result also illustrated that the elongation rate of germ tubes was slower at the higher chemical concentrations. The germination of *M. oryzae* conidia was only inhibited at higher concentrations; similarly as with impedance of germ tube growth. Currently, Azoxystrobin in Australia has no formal registration or recommended dose for controlling rice blast.

Figure1. Effect of Azoxystrobin on germ tube elongation.



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POSTER 44

Genetic resistance of *Eucalyptus* clones to *Ceratocystis fimbriata* strainsR. Reyna^{AB}, M.J. Wingfield^C, J. Roux^C, T. Letsoalo^C, J. Dutour^B, O. Bentancur^D, and C.A. Pérez^A^ADept. Protección Vegetal, EEMAC, FAGRO, Universidad de la República, Ruta 3 km 363, 60000, Paysandú, Uruguay^BUPM - Forestal Oriental, 18 de Julio 818, 60000, Paysandú, Uruguay^CFABI, University of Pretoria, Pretoria, 0028, South Africa^DDept. Biométrica, Estadística y Computación, EEMAC, FAGRO, Universidad de la República

INTRODUCTION

Ceratocystis wilt, caused by strains of a *Ceratocystis* sp., closely related to *Ceratocystis eucalypticola* in the *Ceratocystis fimbriata* species complex (de Beer et al., 2014), represents a serious threat to *Eucalyptus* plantations in Uruguay. The pathogen was first recorded in the country in 2001 (Barnes et al. 2003). In Brazil, it represents one of the most important diseases in clonally propagated *Eucalyptus* plantations. However, in Uruguay, only sporadic epidemic outbreaks have been observed in *E. grandis* plantations. Very little is known regarding the epidemiology or management of this disease in Uruguay. The aim of this study was to determine the value of genetic resistance to control this disease in intensively managed plantations.

MATERIALS AND METHODS

Fungal isolations and preliminary screening for aggressiveness. A collection of 17 isolates of a *Ceratocystis* sp. in the *C. eucalypticola* clade was isolated from infected *Eucalyptus* trees in Uruguay. Each isolate was inoculated on ten 4-month-old seedling plants (stem diameter ~ 5 mm), with a spore suspension injected with a sterile dissection needle into the stems about 10 cm above the soil level as described by Baker et al. (2003). Lesion lengths were determined 2 weeks after inoculation. Ten plants were inoculated with sterile water as a control.

Clonal screening. Five of the most aggressive isolates were used to evaluate the genetic resistance of nine *Eucalyptus* clones. The seedling source used for the initial test for aggressiveness was included for comparative purposes. Five 4-month-old plants were inoculated with each isolate. The lengths of the resulting lesions were determined 8 weeks after inoculation. Five plants of each clone were inoculated with sterile water to serve as a control.

RESULTS

Preliminary screening of isolates. All isolates caused tissue discoloration, however only 4 of 17 showed lesions significantly longer than the controls (Table 1).

Clonal screening. Significant differences ($P < 0.05$) in lesion size were observed among the clones tested (Figure 1). No interaction was found between isolate and clone.

DISCUSSION

Results of this study showed that isolates of *Ceratocystis* collected from dying *Eucalyptus* trees can be highly pathogenic but differ in their aggressiveness. Clones tested with the most aggressive isolates also differed markedly in their susceptibility to infection. This implies that a small number of isolates could be used in order to select clones for resistance to *Ceratocystis* wilt in commercial plantings.

In this preliminary study, there was no evidence for clone by genotype interactions. Such interaction has been shown for other important *Eucalyptus* diseases in the past (van Heerden et al., 2005) and this issue requires further consideration. However, the significant differences in lesion

lengths found among clones, provides good evidence for the potential to use genetic resistance in genotypes as a robust tool for disease management.

Table 1. Lesion length (cm) on 4-month-old seedlings, 2 weeks after inoculation

Isolate*	Lesion length (cm)**	
Control	0,27	a
8318	4,52	ab
8289	4,7	ab
827011	5,66	ab
827912	7,46	abc
8317	7,55	abc
CS5i	8,81	abc
CS3c12	9,85	abc
CS515	9,9	abc
83110	10,87	abcd
P5d	10,93	abcd
P2d	11,94	abcd
CS6c12	12,01	abcd
CS315	13,14	abcd
827111	15,54	bcd
827112	18,14	bcd
M1c	21,05	cd
CA1-3	25,36	d

(*) Isolates in bold were selected for resistance screening

(**) Mean separated by Tukey ($P = 0.05$).

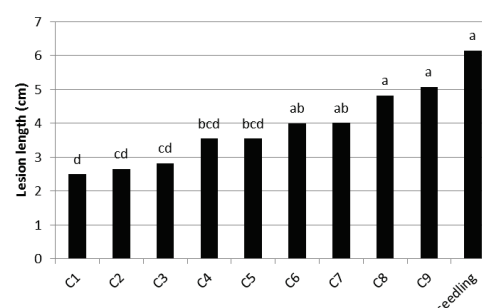


Figure 1. Lesion lengths (average of 5 isolates and 6 replicates each) 8 weeks after inoculation of *Eucalyptus* clones. Letters indicate mean separation based on Tukey ($P = 0.05$).

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POSTER 45

Fertiliser effects on *Pratylenchus thornei* reproduction in pots of chickpea and wheat

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INTRODUCTION

Chickpea (*Cicer arietinum*) is a major pulse crop grown worldwide and susceptible to the root-lesion nematode *Pratylenchus thornei*. Our aim was to improve the methodology used for screening chickpea for *P. thornei* resistance by investigating effects of different fertiliser regimes in combination with Rhizobium bacterium (*Bradyrhizobium*, Group N) on the reproduction of *P. thornei* in chickpea and wheat grown under glasshouse conditions.

MATERIALS AND METHODS

Single plants were grown for 18 weeks under controlled glasshouse conditions in 330 g pots of black semi pasteurised (80°C) Vertosol soil using a bottom watering system (1). Eight chickpea lines with a range of *P. thornei* susceptibility and two wheat check lines, cv. Petrie (susceptible), and cv. QT8343 (partially resistant) were tested. The three fertiliser regimes were mixed through soil before planting and consisted of (1) No fertiliser (2) Osmocote rate 1 g/pot slow release fertiliser (3) solution based nutrients consisting of calcium nitrate, potassium sulphate, sodium dihydrogen orthophosphate and zinc chloride. Each fertiliser treatment was tested with and without Rhizobium. Rhizobium was applied in a slurry onto seed at planting and *P. thornei* added at planting at the rate of 10 *P. thornei*/g equivalent of oven dried soil.

A linear mixed model approach was used to analyse the data, using the REML procedure in GenStat (17th edition) with level of significance set at $P=0.05$. Nematode data was transformed by $\log(P. thornei / \text{kg})$.

RESULTS AND DISCUSSION

Results showed plant height, maturity and plant dry weight all had significant interactions for genotype x fertiliser. Plant height was lower in the no fertiliser treatment for all chickpea lines with exception of Kyabra; wheat height was not affected. Osmocote slowed plant maturity for chickpea cv. Flipper however, in the wild relative ILWC 246, maturity was quicker with Osmocote. Overall results for plant dry weight showed that having no fertiliser resulted in lower weights.

Effects of treatments on *P. thornei* reproduction showed a significant reaction between genotype x fertiliser (Fig1). Significant interactions were also shown for rhizobium x fertiliser these results are shown in Table 1.

Based on these results future screening of chickpea for resistance to *P. thornei* will be with the use of fertiliser in solution with rhizobium as this combination resulted in greater *P. thornei* numbers as opposed to using Osmocote where numbers were generally lower.

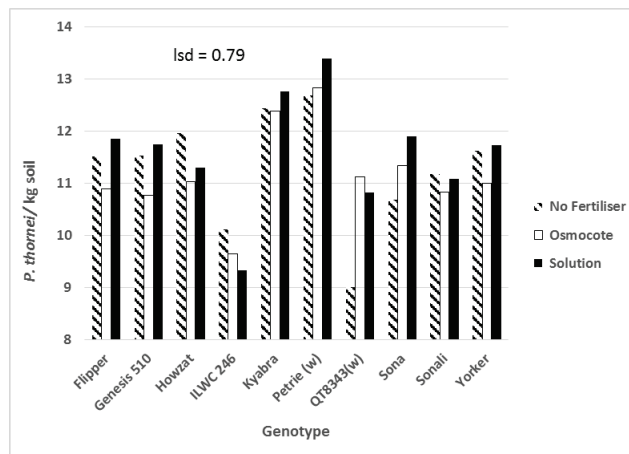


Figure 1. The significant interaction of fertiliser x genotype ($P < 0.001$) on *P. thornei* reproduction in pots of chickpea and wheat (w) = wheat genotype.

Table 1. Mean *P. thornei* numbers resulting from effect of fertiliser x Rhizobium interaction ($P < 0.027$). Rhizobium combined with fertiliser solution gave significantly higher *P. thornei* numbers. Treatments with the same subscript are not significantly different

Rhizobium	Fertiliser	<i>P. thornei</i> /kg soil ln(x)
Rhizobium	Solution	11.78 a
No Rhizobium	Solution	11.41 b
No Rhizobium	Osmocote	11.35 bc
Rhizobium	No Fertiliser	11.31 bc
No Rhizobium	No Fertiliser	11.25 bc
Rhizobium	Osmocote	11.03 c

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POSTER 46

Biotic and abiotic factors predisposing marri (*Corymbia calophylla*) to canker disease caused by *Quambalaria coyrecup* in the southwest of Western Australia

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INTRODUCTION

The decline of *Corymbia calophylla* (marri) has been increasing over recent years. A canker caused by the fungus *Quambalaria coyrecup*, believed to be endemic to WA, has devastated many marri stands in the southwest of WA (Paap et al. 2008). The progression of the decline strongly suggests a breakdown in the ability of the trees to maintain nutrient balance and we believe that mycorrhizal fungi play a role in this process. Mycorrhizal fungi are known to be more important for the uptake of vital nutrients on low fertility sites and are essential for below-ground carbon storage; thus, any change in the mycorrhizal community would result in critical changes in nutrient uptake and tree health as evidenced by declining crown vigour (Scott et al. 2013). It is possible that the use of pesticides and herbicides are causing a decline in the diversity of mycorrhizal fungi in disturbed sites, and thus predisposing marri to canker.

MATERIAL AND METHODS

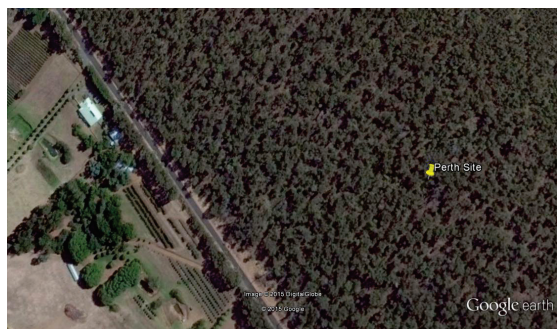


Fig. 1. Field site showing transect from highly disturbed (remnant stand where incidence of canker is highest) to healthy (middle of forest block).

To develop a theoretical approach, a site in Pickering Brook, WA was intensely surveyed in 2014. The site consisted of a gradient from a highly disturbed area (road edge along cleared land) to healthy forest block (opposite side of road; Fig. 1). The gradient included 5 lateral transects consisting of 10 trees each. Site characteristics were measured: leaf litter depth, canopy health, diameter at breast height, and incidence of canker disease.

Soil was collected from each tree along each transect and tested for nutrient composition. Fine roots were collected from these soil samples to determine the mycorrhizal composition. DNA was extracted from fine roots and amplicon pyrosequencing conducted using primers ITS7A & ITS4, commonly used for fungal microbial community analysis in soils. High throughput sequencing (HTS) was carried out on GS Junior 454 platform (Roche) to determine the community composition of mycorrhizal fungi (and other higher soil fungi).

RESULTS

A total of 185, 173 sequences passed quality control. The average length of a read was 293. We got a total of 1469 operational taxonomic units (OTU's) from the pyrosequencing run and 843 singletons. The average read for each molecular identifier (MID) used was 3388.

Table 1. Results from a one-way ANOSIM showing that the fungal communities in the disturbed transect (1) are significantly different from all other transects (i.e., 2-5 are located on the opposite side of the road in intact forest).

1	2	3	4	5
1	0.0006	0.0005	0.0011	0.0339
2	0.0006	0.2542	0.3163	0.0835
3	0.0005	0.2542	0.2428	0.2114
4	0.0011	0.3163	0.2428	0.5515
5	0.0339	0.0835	0.2114	0.5515

DISCUSSION

Our preliminary results demonstrate that fungal communities in the disturbed region are significantly different from fungal communities in the intact forest. This could be one of the predisposing factors that are making marri more susceptible to canker disease along these roadside edges. Currently, identification of the OTU's is being undertaken to determine their likely function and reveal what fungal species are causing the observed differences between sites.

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POSTER 47

Efficacy of chlorine, chlorine dioxide and ultraviolet radiation as disinfectants against waterborne pathogens in irrigation water

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INTRODUCTION

Water used for irrigation in plant nursery and horticultural production systems is frequently captured for reuse. This conserves water, protects the environment from nutrient and pesticide runoff, and reduces production costs associated with purchasing water. However, reusing water in production systems can increase the risk of plant disease (1). A number of disinfection treatments are available to reduce the risk of plant disease when reusing irrigation water, however, limited studies have compared the efficacy of these disinfection treatments on a range of plant pathogen species, on their various life stages and in different water qualities (2). The objective of this study was to assess the efficacy of chlorine, chlorine dioxide and UV-C radiation against different propagules of eight common plant pathogens in two water qualities.

METHODS

In this study, propagules from eight plant pathogens including *Clavibacter michiganensis* subsp. *michiganensis*, *Alternaria alternata*, *Chalara elegans*, *Colletotrichum gloeosporioides*, *Calonectria pauciramosa*, *Fusarium oxysporum*, *Phytophthora cinnamomi* and *Pythium aphanidermatum* were exposed to chlorine (as sodium hypochlorite) at 0, 1, 2, 5 ppm for 0, 10, 20, 30 minutes; chlorine dioxide at 0, 1, 3, 5 ppm for 0, 4, 8, 10 minutes; and ultraviolet radiation (UV-C) at 100 mJ/cm² and 250 mJ/cm². Disinfection of the propagules of each pathogen were tested in both deionised water and dam water. Viability of pathogen propagules after treatment was assessed using viability plating.

RESULTS

The efficacy of chlorine, chlorine dioxide and UV radiation, varied with exposure time, application rate, water type and the pathogen and propagule. All three disinfection treatments applied significantly affected propagule viability ($P < 0.001$) at all rates and times. The effect of chlorine and chlorine dioxide application rate and exposure time on the disinfection of different pathogen propagules was variable. *C. michiganensis* subsp. *michiganensis*, *P. cinnamomi* and *P. aphanidermatum* propagules were most sensitive to all treatments, while propagules of *Ch. elegans*, *Ca. pauciramosa* and *F. oxysporum* required the highest rates and exposure times to chlorine, chlorine dioxide and UV for complete disinfection. The application rates and times required to kill pathogen propagules in dam water were often greater than those required in deionised water (Fig. 1). Chlorine dioxide applied as a 'shock' treatment and UV radiation offered more effective biocidal activity than chlorine in both water types.

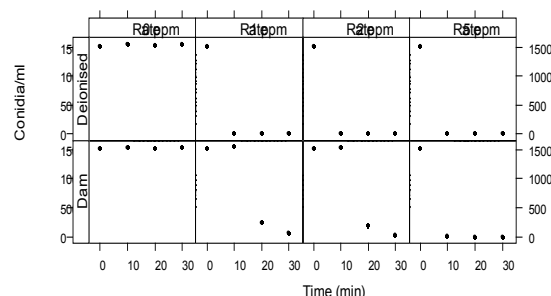


Figure 1. Effect of chlorine (sodium hypochlorite) on *F. oxysporum* conidia in deionised and dam water. Each point is the mean of three replicates $\pm$ the standard error of the mean

DISCUSSION

This study has begun to address the gaps in the knowledge surrounding the effect of disinfection treatments on different life stages, or propagules, of different plant pathogens, and the role of water quality characteristics on efficacy of disinfection. Water quality can vary significantly between locations and the efficacy of disinfection treatments may vary with the quality of the water. We have provided evidence supporting those treatments that are more effective against selected pathogens, and we have shown which propagules of those pathogens are more sensitive to each treatment. This is important when considering the epidemiology of each pathogen in the plant production system. Ultimately, the selection of a disinfection system for irrigation systems will depend on a number of factors including current hygiene practices in the nursery, water quality, plant species grown in the nursery, pathogens present and the resources available to the producer.

ACKNOWLEDGEMENTS

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POSTER 48

Discrimination of fungi associated with grape bunches using attenuated total reflection mid-infrared (ATR-MIR) spectroscopy

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INTRODUCTION

Grape bunches are colonised by a wide range of microbes, many of which can reduce quality of grapes and wines (1). The impact of single pathogenic or opportunistic species on quality of grapes and wines may be hard to determine due to the species diversity on field-grown bunches in grape growing regions and climatic zones (e.g. *Erysiphe necator* and *Aspergillus* spp.; *E. necator* and *Botrytis cinerea*).

E. necator is a ubiquitous, obligate pathogen occurring in all climatic zones, and may be associated with the opportunistic pathogen *B. cinerea*, which causes botrytis bunch rot (BBR), if conditions are favourable towards the end of ripening. Consequently, fungal colonisation may have detrimental effects on bunches resulting in the rejection of grape loads by wineries.

ATR-MIR spectroscopy enables non-destructive, rapid and simple analysis of samples. It has been used for estimation of BBR severity, quality control of crops and chemical characterisation and identification of microbes to genus, species and/or strain level.

The aim of this research was to examine the suitability of using ATR-MIR to discriminate fungi commonly associated with grape bunches.

MATERIALS AND METHODS

Fungal isolates and identification Isolates of two obligate pathogens (*E. necator* n=2; *Plasmopara viticola* n=1) from grape leaves (4 replicates/isolate) and four facultative fungi, *Aspergillus tubingensis* (n=1), *A. carbonarius* (n=1), *Botrytis cinerea* (n=2) and *Penicillium chrysogenum* (n=1) from grape bunches (5 replicates/isolate) were examined. Morphological identification was confirmed by DNA sequencing of the ITS and β -tubulin regions.

ATR-MIR analysis Isolates of facultative organisms were grown and processed using methods modified from Lecellier et al. (2014) (2). Suspensions of *E. necator* conidia and *P. viticola* sporangia were prepared by gently shaking sporulating Sultana leaf pieces in sterile water. Mycelial and spore pellets of facultative and obligate organisms were resuspended in 0.9% NaCl, respectively. Suspensions (5 μ l) were deposited on the ATR cell and scanned in the MIR range (1800-800 cm^{-1}) (Bruker Alpha). Principal component analysis (PCA) was used to determine relationships within facultative and obligate organisms independently. Partial least squares discriminant analysis (PLS-DA) was used to classify isolates.

RESULTS AND DISCUSSION

E. necator and *P. viticola* were dissimilar in composition (Fig. 1), reflected in spectral differences at 1044 cm^{-1} (polysaccharides), 1075 cm^{-1} (sugar moieties) and 1169 cm^{-1} (phospholipids). Accordingly, they did not cluster together in the PCA score plot (data not shown).

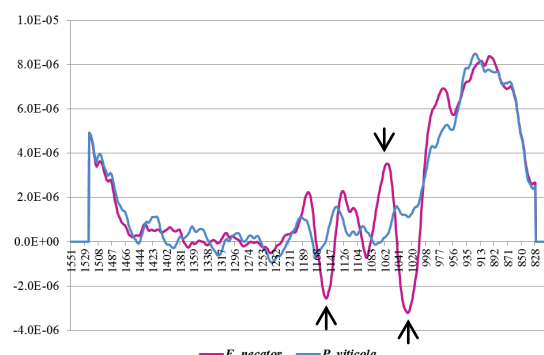


Figure 1. Second derivative of the ATR-MIR spectra of *E. necator* and *P. viticola*. Spectral differences are indicated by arrows.

Separation of the four facultative species was achieved with PC1 and PC3, which explained 42% of the variation in the data set. Major spectral difference between ochratoxin producer *A. carbonarius* and the remaining species was in the lipid region (1445-1455 cm^{-1}), indicating that lipids might play a role as signals for regulating the biosynthesis of toxins.

The classification success rate based on PLS-DA was 100% for *A. carbonarius* and *A. tubingensis*, 90% for *B. cinerea* and 60% for *P. chrysogenum*. Overall, it is possible to achieve robust classification of four common grape bunch colonisers with an 88% success rate when ATR-MIR is applied.

Further research is required to validate MIR-ATR classification methods for facultative and obligate organisms.

ACKNOWLEDGEMENTS

This research was conducted with support from the Australian Grape and Wine Authority.

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POSTER 49

The role of woodborers on the spread and infection of Ophiostomatoid fungi in marri and jarrah

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INTRODUCTION

Mediterranean type forest ecosystems commonly experience an extended dry period during summer months. In recent decades many Mediterranean regions have experienced elevations in temperature and decreases in rainfall, which have led to large-scale forest die-off events. This has also recently occurred in the Northern Jarrah Forest (NJF) (Matusick et al. 2012) and was closely associated with increased infestations by borers. These borers caused severe damage to jarrah and marri trees and contributed to increased tree stress and tree mortality.

Blue-stain (Ophiostomatoid) fungi, which attack woody tissue, can be closely associated with bark beetles. These fungi have been found in *Eucalyptus* species, invading the sapwood (van Wyk et al 2007), and appear to be present in the jarrah forest of Western Australia.

In this study, the occurrence and relationships between borers and blue-stain fungi in jarrah forest under drought conditions, is being investigated.

MATERIALS AND METHODS

Sites were chosen in the NJF where trees had experienced several die-off events over the last 5 years. Trees were sampled that showed canopy dieback with dead foliage remaining on the trees and in close association with healthy trees. Also logs were harvested and left in the field for sufficient time to allow infestation by borers.

Sampling involved removal of bark sections (10x10cm) to reveal the sapwood surface. The presence of woodborer activity, and whether staining of sapwood had spread from galleries was recorded. Along each gallery, three points were selected and gallery width and distance of stained sapwood at right angles to the gallery measured. Sapwood samples were taken from stained areas on trees and logs and used to isolate and identify fungi.

RESULTS

There was an association between borers and fungal stain in tree and log samples for approximately 50% of samples taken.

Staining by fungi were observed in the sapwood, starting close to the egg oviposition site, and then spreading throughout the sapwood in association with the galleries as they radiated out from this point. For example, on a stressed jarrah tree, the area of staining doubled for each 2-fold increase in distance from the oviposition site, with borer galleries spreading at about 35 degrees (Figure 1).

Preliminary results indicate staining in borer galleries occurs more often in logs of marri compared to jarrah. In logs of marri the stained region was 3.84 ± 0.36 times wider than the width of the average borer gallery width (Table 1).

Table 1. Gallery and staining width in marri logs (SE=standard error of the mean).

Variable	Width $\pm$ SE (mm)
Gallery	4.40 ± 0.35
Sapwood stain	16.90 ± 0.96

Some structures found on sapwood appear to be of similar morphology to blue-stain fungi. Isolated colonies have yeast like appearance that closely resembles the *Sporothrix* anamorph. More detailed examination of structures, culture characteristics and molecular analysis are currently underway to determine the type and species of blue-stain fungi associated involved.



Figure 1. Egg oviposition site and spread of fungus on a standing jarrah tree. Arrows shows extent of stained area associated with borer galleries.

DISCUSSION

Results indicate the fungi may be transported by borers between trees, or the galleries made by borer larvae may provide preferential pathways for the spread of fungi and area of infection throughout the tree. Understanding the interactions between borers and native blue-stain fungi in marri and jarrah trees, subjected to drought, may contribute to our understanding of factors causing *Eucalyptus* trees to die when stressed. Further work is now underway to clearly define the diversity of native blue-stain fungi within the NJF and their association with borers.

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POSTER 50

Pyramiding yellow spot resistance loci into fixed lines results in genetic gain

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INTRODUCTION

Although good progress has been made internationally to understand yellow spot (YS), caused by *Pyrenophora tritici-repentis*, resistance in wheat, relatively few resistance genes have been identified and mapped in Australian germplasm. Only one (*tsn1* on 5BL) is in general and known use in Australian breeding programs. Under a current national project a major effort has been made to improve the understanding of genetics of YS resistance in present and future donors, identify novel quantitative trait loci for resistance and develop a series of fixed lines, each carrying YS resistance genes from 2 or 3 resistance sources in elite Australian backgrounds that can be used as parents in resistance breeding.

MATERIALS AND METHODS

Nine doubled haploid mapping populations (five of which were fixed for *tsn1*) were screened for YS resistance at various growth stages, environments and national sites. Five of these populations were genotyped by the Australian Wheat and Barley Molecular Marker Program and nine new resistance loci were mapped. Resistance loci on 2A and 1AS (Fig. 1) were stacked along with *tsn1* on 5BL using single seed descent. Resistant parents were selected using both phenotypic and genotypic data and a cross was made in the spring of 2011. F₂s were progressed through to F₄ while being subjected to marker assisted selection. Thirty two F₅ lines were developed which are triple homozygotes at the three YS resistance loci and are also fixed for the *vrn-1* locus. These fixed lines are both in short season and long season backgrounds and were phenotyped at various growth stages and environments.

RESULTS

Adult plant field response to yellow spot infection in 16 F₅ lines with stacked resistance loci is presented as blue bars in Fig. 2. Orange bars are individual selections from the two populations which were used in crossing for gene stacking while the red and green bars are the parents of the two DH populations. Similar results were obtained at the seedling stage and adult plant stage under controlled environment. Some very resistant individuals amongst the F₅ lines indicate significant genetic gain as compared to the parents and grandparents.

DISCUSSION

Genes for yellow spot resistance can be successfully combined into fixed lines using single seed descent and marker assisted selection. Fixed lines with stacked resistance genes express significantly higher resistance at both seedling and adult plant stages that is effective in various environments. These lines are important resources that can be used by breeders for rapid development of varieties with high levels of resistance. Newly developed resistant varieties will greatly reduce the estimated annual losses of \$212M per annum caused by this disease in Australia (Murray and Brennan 2009).

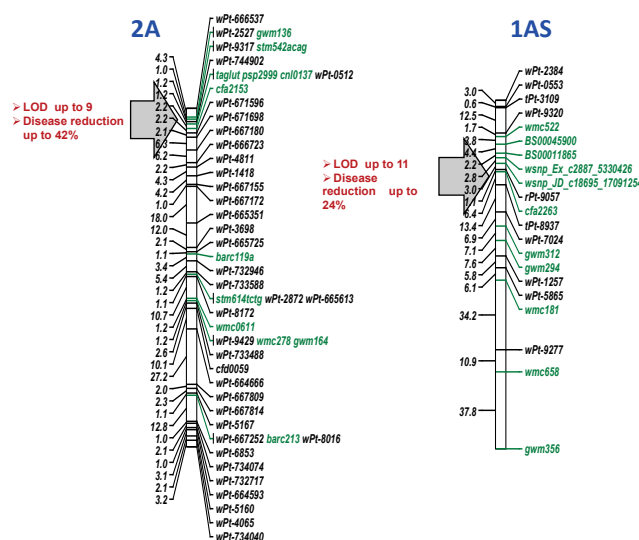


Fig. 1. QTL for yellow spot resistance on 2A in Calingiri/Wyalkatchem and 1AS in IGW2574/Annuello. Markers in green font are SSRs, STSs and SNPs and the ones in black are DARTs.

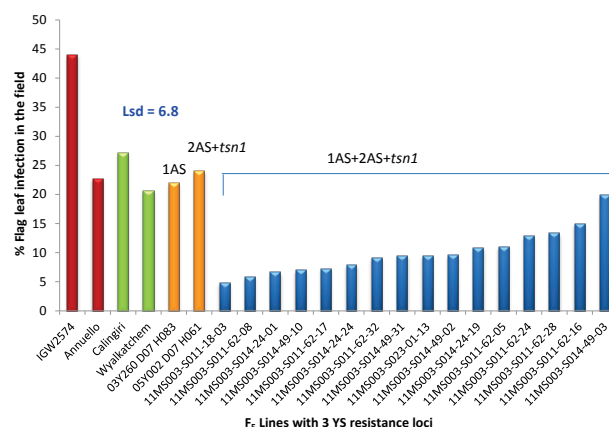


Fig. 2. Adult plant field response to yellow spot infection in 16 F₅ lines with stacked resistance loci.

ACKNOWLEDGMENT

This research is supported by the Grains Research and Development Corporation (GRDC).

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POSTER 51

A modified method for *Pyrenophora teres* f. *maculata* spore production

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INTRODUCTION

Pyrenophora teres f. *maculata*, the causal agent of spot form of net blotch, is a major stubble foliar disease of barley in Australia and internationally (1). Resistance screening assays are routinely conducted in the glasshouse and are reliant on production of inoculum consisting of conidia and/or mycelia suspensions (2). The use of conidia is preferred to mycelia suspensions as they are quantitative and better reflect field conditions.

Lamari and Bernier (3) proposed a method for *Pyrenophora tritici repentis* spore production that included temperature reduction at the conidia formation phase.

This study investigated the effect of temperature at the conidia formation stage on conidia production of *P. teres* f. *maculata*.

MATERIALS AND METHODS

Four isolates of *P. teres* f. *maculata* (Table 1) were cultured on PDA agar. From these stock cultures two plugs (2 mm × 2 mm) were subcultured onto a 90 mm V8 agar petri dish. Each isolate was replicated three times and incubated at 20 ± 1°C for seven days with a 12 hour photoperiod. Isolates were then placed into darkness at 4, 8, 12 and 16°C for 24 hours during the conidia production phase. To harvest spores after the 24 hours incubation, 10 mL of water was added to each plate and spores washed into a suspension. The concentration of conidia in the suspension was determined by aliquoting 15 µL onto a standard glass slide and visually counting spores. Average spores produced per plate were calculated, transformed (Ln x + 1) and analysed using ANOVA in GenStat (14th edition).

RESULTS

Temperature significantly affected spore production of one (SF 33/12) of the four isolates tested with 16°C producing the most spores. The second highest spore production was

observed at 4°C but did not significantly differ from 8°C or 12°C.

SF 33/12 produced the most spores overall with an average production per plate of 16,531 spores. The two isolates with the lowest spore production produced less than 1,000 spores per plate on average.

DISCUSSION

The number of spores produced varied depending on the isolate and temperature with isolate selection influencing spore production the most. Based on the number of isolates used in this study, and the variation in spore production across these isolates, further study is required to determine whether temperature effects on spore production show a trend. The isolate with the highest overall spore production (SF 33/12) appears to have an optimum temperature of 16°C which is consistent with *P. tritici repentis* (M. Shankar pers comm 2011, DAFWA). Microscopic examination of some of the cultures after the eight days of incubation, showed a large number of conidiophores with no conidia present. Indicating a potential for higher spore production. Further method modification is required to achieve this.

ACKNOWLEDGMENTS

We would like to thank the Grains Research and Development corporation and the Victorian government for funding.

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Table 1. Average spore production of four *Pyrenophora teres* f. *maculata* isolates in vivo at different temperatures during the conidia development stage.

	Number of spores per plate (back transformed means)				
	Isolate				
Temp	07-047	ptm12-026	ptm13-134	SF 33/12	Mean
4°C	877 a ^A	1,761 ab	945 a	20,888 cd	6,118 b ^B
8°C	341 a	2,965 ab	543 a	9,451 bc	3,325 ab
12°C	933 a	3,494 ab	1,188 a	2,726 abc	2,085 a
16°C	546 a	4,141 abc	1,100 a	33,056 d	9,711 c
Mean	674 a ^C	3,090 b	944 a	16,531 c	

^A Temperature by isolate means followed by the same letter are not significantly different (P<0.05)

^B Temperature means followed by the same letter are not significantly different (P<0.05)

^C Isolate means followed by the same letter are not significantly different (P<0.05)

POSTER 52

A comparison of the efficiency of gene silencing in sap sucking and chewing insects using different expression systems to deliver dsRNA

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ABSTRACT

Infection of crop plants with pests and diseases cause both direct and indirect damage to crops, and pose a serious threat to agriculture. The Green Peach Aphid (sap sucking) and the cotton bollworm (chewing) are widely distributed herbivorous insects that are a major threat to horticulture and broad acre agriculture in Australia: both cause direct and indirect damage to crops. Effective eradication of these pests is hard to accomplish because they are becoming resistant to many of the current insecticides and hence new control techniques are needed. RNA interference (RNAi) technology is a powerful approach in which the presence of double stranded RNA (dsRNA) in a cell signals any messenger RNA (mRNA) with the same sequence for degradation, so preventing or silencing its expression. Transgenic plants expressing dsRNA of target genes in insects is an approach to generate a continuous supply of dsRNA to the feeding insects. Current research shows that RNAi delivered through crop plants engineered by modifying their nuclear genomes can be limited rather than complete. For efficient RNAi effects in insects, dsRNAs greater than or equal to approximately 60 base-pairs are required, but plants have their own RNAi pathway producing small interfering RNAs, which prevents accumulation of long dsRNA. Plastids the cell organelles which lack an RNAi pathway, and so provide a mechanism to deliver unprocessed long dsRNA to pests. The question of whether different genome expression approaches can generate 100% control of these pests will be answered by this project.

POSTER 53

Comparison to apply antagonists on the surface with warm water and Thiabendazol treatments to control green mold causes by *Penicillium digitatum* on Mexican lime fruits

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ABSTRACT

Green mold caused by *Penicillium digitatum* is an important and destructive storage disease of citrus fruits. Recently, biological control methods of storage diseases are getting more prevalent due to the effects of fungicide residues on fruits.

According to statistical analysis the power of inhibition these two antagonists to compare with warm water and Thiabendazol, fungicide was the first level, antagonists were the second level and warm water (45°C) was in the third level.

Because of wilting in some fruits after thermotherapy and risks of chemical method like fungicide residue and problems arising to pathogen resistance, to achieve alternative and safe methods like biological control is necessary.

In this research after sterilizing fruits by ethanol, first dipping in spore suspension (10^6 spores ml⁻¹) then air dried them and immersed in antagonist suspension, warm water and fungicide. Treated fruits were stored at 24°C and 90-95% RH.

POSTER 54

Are wounds necessary for *Botryosphaeria* infections in blueberries?

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INTRODUCTION

Botryosphaeriaceae species cause damaging canker and dieback symptoms in a many perennial fruit crops [1]. In blueberry, several *Neofusicoccum* spp. are responsible for Botryosphaeria stem canker, twig blight and dieback. Several studies have indicated that infection by the splash-borne conidia of these species is likely to be through susceptible wounds. However, one study [2] showed that *Fusicoccum aesculi* spore penetration could also take place through open stomata in blueberry stems. The following experiments determined whether wounds were necessary for infection of blueberry tissues by three *Neofusicoccum* spp.

MATERIALS AND METHODS

Two year old potted blueberry plants variety Dolce blue were used for these experiments, with six plants per treatment arranged in completely randomised design.

In the first experiment mixed isolate conidium suspensions (10^6 /mL) were used for three isolates each for *N. australe*, *N. ribis* and *N. parvum*. The 10-20 bunches of fruits and flower buds (wounded or non wounded) per plant were each drop inoculated, with 20 μ L of conidial suspension or sterile water (control plants). Tissues were immediately covered with misted plastic bags (48 h). The buds were removed after 10 days and fruits after 14 days for isolation onto potato dextrose agar. The inoculating species were identified by colony morphology after 3-5 days incubation at 25°C in the dark.

In the second experiment wounded and non wounded semi-hard green shoots were drop inoculated (50 μ L) with a mixed conidial suspension (10^6 /mL) of *N. ribis* (three isolates as above) and inoculation points covered with Parafilm®. Plants were then placed inside growth chambers with different relative humidities (RHs) and temperatures (Table 3). After 30 days, assessment was by lesion length and pathogen isolation as described above using 1 cm segments cut from 5 cm above to 5 cm below the inoculation point. Data were analysed by ANOVA using Genstat version 16. Treatment means were compared by Fisher's protected LSD tests at $P \leq 0.05$.

RESULTS

In the first experiment on fruits (Table 1), infection incidence was significantly affected by species ($P < 0.001$), and by wounding ($P < 0.001$) but not by an interaction between species and wounding ($P = 0.897$). For buds, the species effect was also significant ($P = 0.009$) as was wounding ($P < 0.001$). In the second experiment non-wounded stems had small brown blotches at the inoculation sites and lesions only in wounded tissues (Table 2). Lesion length was affected by RH ($P < 0.001$; Table 2) at 20°C but not at 25°C ($P = 0.379$). There was a significant difference ($P < 0.001$; Table 3) in mean % proportion infected in these shoots incubated at 20°C and also between RHs. For plants incubated at 25°C, the mean disease incidence was not significantly different ($P = 0.002$) in wounded and non wounded shoots but was significantly different between RHs.

Table 1. Infection incidence of wounded and non wounded fruits and buds inoculated with *Neofusicoccum* spp.

Species	Fruits			Buds		
	No wound	Wound	Mean	No wound	Wound	Mean
<i>N. australe</i>	46.0	62.0	54.0 a	49.5	60.0	57.8a
<i>N. parvum</i>	57.0	74.5	65.8 b	60.0	75.0	67.5ab
<i>N. ribis</i>	67.0	86.5	76.8 c	66.0	83.0	74.5b

Data in columns with the same letter are not significantly different at $P \leq 0.05$.

Table 2. The lesion length developed in wounded and non wounded shoots incubated at 20°C and 25°C under 90% and 100% relative humidities.

Treatment	Lesion length (mm)			
	20°C		25°C	
	90% RH	100% RH	90% RH	100% RH
Non-wounded	0	0	0	0
Wounded	51.5	26.7	32.0	25.7

Table 3. The % infected proportion in wounded and non wounded shoots incubated at 20°C and 25°C under 90% and 100% relative humidities.

Treatment	Infected Proportion			
	20°C		25°C	
	90% RH	100% RH	90% RH	100% RH
Wounded	95.0	78.0	80.0	60.0
Non wounded	88.3	50.0	61.7	43.3

DISCUSSION

Both wounded and non wounded fruits and buds became infected by all three *Neofusicoccum* spp. but with 18-25% lower incidence in non-wounded tissues. In the second experiment, non-wounded semi-hard green shoots developed no lesions but wounded shoots did, irrespective of the environmental factors used. Both tissues were colonised by the pathogen to similar extents. In non-wounded tissues, symptoms may develop when plants are stressed or dormant, as shown for grapevines [3]. This research has shown that non wounded tissues may also become infected by these pathogens, with a similar potential for disease.

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POSTER 55

How root-lesion nematode (*Pratylenchus thornei*) affects wheat growth and reduces grain yield

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INTRODUCTION

The root-lesion nematode (*Pratylenchus thornei*) is a major pathogen of wheat in Australia. It is particularly damaging in the sub-tropical grain region of northern New South Wales and southern Queensland.

Information is required on the effect of *P. thornei*, on intolerant wheat varieties, at what growth stages it impacts plant growth and how this translates to loss of grain yield. Better knowledge in this regard will help in the modification of crop growth models to simulate the impact of *P. thornei* on wheat yield (1).

Aldicarb is the best nematicide for control of root-lesion nematode for research purposes in the heavy clay soils or Vertosols of the sub-tropical grain region, and has no detectable impact on microbial populations or mineralisation of soil nitrogen (2).

Here we report a field experiment in which rates of aldicarb were used to create a range of population densities of *P. thornei* in order to examine this nematode's impact on the wheat plant's development and yield.

MATERIALS AND METHODS

Field experiment. Five rates of the nematicide aldicarb (0, 0.125, 0.25, 0.5, 1, 2, 4 kg/ha) were applied at sowing with wheat cv. Gatcher in a 3-replicate experiment in a field infested with *P. thornei* at Pirrinun (Lat 27.03°S, Long 151.23°E, elevation 359 m) on the Darling Downs of Queensland. Plots were 20 m long x 7 drill rows 25 cm apart in randomised blocks. At 72 days after sowing (stem elongation or Zadoks growth stage 32) quadrats were taken to determine plant dry weight, nitrogen and phosphorus concentrations of the tops and population densities of *P. thornei* in the roots and root zone soil. Biomass and numbers of heads were determined at anthesis and grain yield, grain weight and grain number were determined at maturity.

RESULTS

Population densities of *P. thornei*. Increasing the rate of aldicarb resulted in decreasing numbers of *P. thornei* in both the roots (data not shown) and root zone soil of the wheat plants at Z32 (Fig 1).

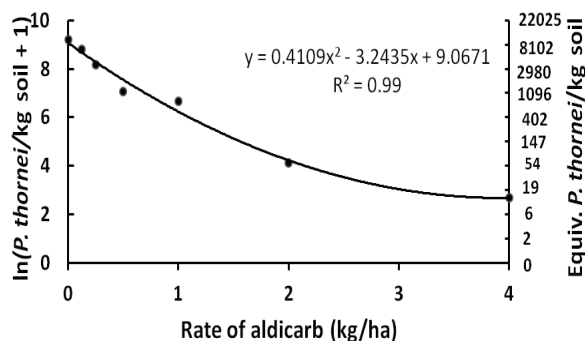


Figure 1. Effects of rate of the nematicide aldicarb applied in the seed row at sowing on *Pratylenchus thornei* in the root-zone soil at Z32 (stem elongation)

Plant parameters. As *P. thornei* population densities were decreased with increasing rate of aldicarb there were significant increases in plant biomass (kg/ha), tiller density (number/m²), biomass/tiller (g), N concentration (%), P concentration (%), N uptake (kg/ha) and P uptake (kg/ha) at GS32, and in plant biomass (kg/ha) and head density (number/m²) at anthesis, and in grain yield (kg/ha) and grain number (no./m²) at maturity. There were highly significant negative quadratic relationships between these various plant parameters and the population density of *P. thornei* in the root zone soil, with R² ranging 0.78–0.93 for n=8.

There was a highly significant negative linear relationship between grain yield and the population density of *P. thornei* in the root zone soil at GS32 (Fig 2).

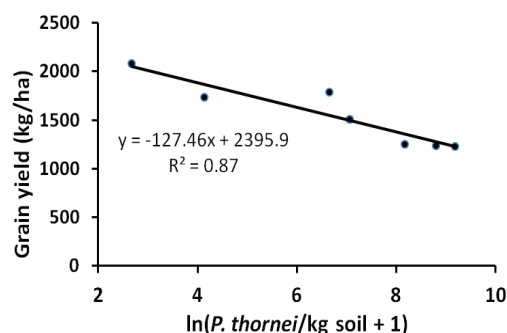


Figure 2. Effects of population density of *P. thornei* produced by different rates of the nematicide aldicarb applied in the seed row at sowing on grain yield

DISCUSSION

P. thornei reduces root function through migrating, feeding and reproducing in the root cortex. Vegetative symptoms of nutrient deficiencies can appear in the tops of plants having their roots attacked by *P. thornei* even though nutrient levels in the soil may be considered adequate. Our results show that *P. thornei* causes lower concentrations of N and P in the tops, reduced tillering and biomass production and lower head density. These effects result in reduced number of grains/unit area. It is through this reduction in grain number that *P. thornei* causes grain yield loss rather than through reduction of grain size.

ACKNOWLEDGEMENTS

We thank GRDC for funding and Mr E. Morris for provision of land.

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POSTER 56

A new tamarillo powdery mildew record from New Zealand

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INTRODUCTION

Several powdery mildew fungi are known to infect tamarillo trees (*Solanum betaceum*). In New Zealand, the following powdery mildew species have been recorded from tamarillo: *Golovinomyces cichoracearum*, *G. orontii* and *Erysiphe polygoni*. From other parts of the world *Leveillula taurica* and *Pseudoidium neolycopersici* have also been identified (1, 2). In November 2014, a suspected new tamarillo disease was reported to the Ministry for Primary Industries. Symptoms included a white mycelial layer, yellowing and curling of leaves, and premature defoliation. Morphological and molecular identification was carried out to determine the causal agent of this powdery mildew disease.

MATERIALS AND METHODS

Two diseased tamarillo leaf samples were obtained from residential gardens on the North Island of New Zealand. The first one was collected in December 2014 from Mt. Maunganui and the second one in January 2015 from Auckland. Both samples were dried and vouchered in the New Zealand Fungal and Plant Disease Collection under the accession numbers PDD 107432 and PDD 107433, respectively.

Morphological characterisation was completed with a Zeiss Axio Imager A1 light microscope using objectives with 40× and 100× magnification. For molecular identification, infected plant tissue was homogenised using the MagNA Lyser Green Beads tubes (Roche). Genomic DNA was extracted with an InviMag Plant DNA Mini Kit (Invitex) and the KingFisher extraction system (Thermo Scientific). The ITS region was amplified with primers PMITS1 and PMITS2. PCR products were sequenced by EcoGene (Auckland, New Zealand) and sequence analysis was performed in Geneious 7.1.5. Maximum likelihood phylogenetic analysis (ML) was completed via Cypres web portal using the RAxML-HPC2 on XSEDE tool.

RESULTS AND DISCUSSION

The morphology of the powdery mildew was characterised as follows: mycelium amphigenous, thin, whitish; hyphae branched, septate; conidiophores long, straight, erect, with very long primary or secondary foot-cells (70–115 µm); conidia ellipsoid to ovoid, 29–36 × 12–17 µm (average 15 × 33 µm). Morphological comparison using Braun and Cook (2) indicated that this pathogen is a member of the anamorphic genera *Oidium* or *Euoidium*. However, identification to species level was not possible because the morphological characters overlapped with a few different species.

BLASTn analyses of the ITS sequences obtained in this study (KR105772 and KR105774) were completed via NCBI GenBank. A 100% identity was determined to five *Euoidium longipes* (syn. *Oidium longipes*) sequences (e.g. AB769440) and 99% identity (with 1–2 bp differences) to six other *E. longipes* sequences (e.g. HQ286675). Although two *E. lycopersici* sequences (EU327330, EU327331) were also found to be identical to our sequences, these comparisons are only partial and do not include the variable poly-T motif

which is the molecular character differentiating *E. longipes* and *E. lycopersici* (3).

ML analysis of the sequences from tamarillo, a selection of nearly identical collections, and representatives of other powdery mildew species reported from tamarillo confirmed that the new collections from New Zealand are different from all the other previously known tamarillo powdery mildew records. However, it did not enable differentiation between the *E. longipes* and *E. lycopersici* isolates (Fig. 1). Therefore, the differentiation between these two species still needs to be completed by comparing the poly-T region until sequence data from more variable gene regions become available.

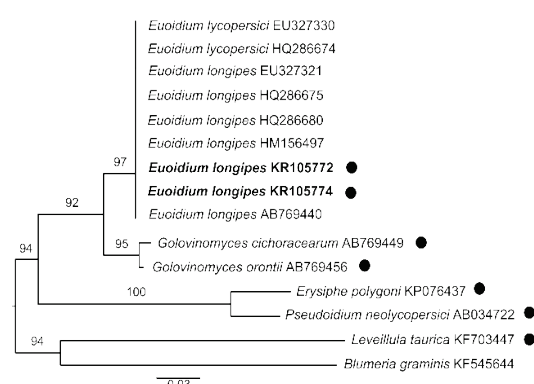


Figure 1. ML tree showing phylogenetic relationships between the *E. longipes* from tamarillo leaves in New Zealand (in bold type), some earlier *E. longipes* and *E. lycopersici* collections from different hosts, and the other powdery mildew species recorded from tamarillo (●). Topology is rooted with *B. graminis* and branch labels represent bootstrap values.

Previously known hosts of *E. longipes* include *Nicotiana* sp., *Petunia × hybrida* and *Solanum melongena*, and to date, the species has been identified from Europe and North America (2, 3, 4). Therefore, this is the first record of *E. longipes* in New Zealand and on tamarillo worldwide. Additional studies of powdery mildews on solanaceous hosts in New Zealand are needed to further determine the distribution and host range of *E. longipes*. Molecular studies are also needed to verify whether the other morphology-based records of powdery mildews on tamarillo are accurate and to better understand the diversity of powdery mildews on tamarillo.

ACKNOWLEDGEMENTS

We thank Janine Mayes and Rudi Schnitzler for collecting the tamarillo leaf samples, and Stephanie Keeling for completing the DNA extractions and amplification for molecular identification. Rob Taylor and Lia Liefteing are acknowledged for peer-review.

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POSTER 57

Characterisation of *Fusarium oxysporum* f.sp. *niveum* by race differentials and molecular MarkersL.T.T. Tran-Nguyen^A, S.E. Cook^A, B.D. Condé^A, C.A. McMaster^A, V. Puno^B, K.L. Everts^C, L. Tesoriero^D, E. Liew^E and D. Guest^B^APlant Industries, NT Department of Primary Industry and Fisheries, GPO Box 3000, Darwin, 0801, NT, Australia^BFaculty of Agriculture and Environment, The University of Sydney, NSW 2006, Australia^CUniversity of Maryland and University of Delaware, 27664 Nanticoke Rd Salisbury, MD 21801 USA^DNSW Department of Primary Industries, Locked Bag 26 Gosford NSW 2250, Australia^EBotanic Gardens & Centennial Parklands, Mrs Macquaries Road, Sydney, NSW 2000, Australia

INTRODUCTION

Fusarium wilt is a severe disease of watermelon and is caused by *Fusarium oxysporum* f. sp. *niveum* (*Fon*). The disease is a major yield limiting factor in production worldwide, and was first detected in the Northern Territory (NT) in Australia in May 2011. The disease affected triploid watermelon seedlings and plants (1). Symptoms included leaf necrosis, necrotic blotching and seedlings death. *Fon* can be divided into four races (0, 1, 2 and 3). To date, two of the races have been detected in Australia however there is limited information on these *Fon* races. *Fon* races are differentiated according to their aggressive variability on differential cultivars (2,3). NT isolates were confirmed to be *Fon* with a PCR test (4). However, the *Fon* specific test did not differentiate races. Our objectives were to determine the race of the NT *Fon* isolates by conducting race differential trials and improve the diagnostic test for *Fon* by targeting the putative effector secreted in xylem (SIX) gene(s).

MATERIALS AND METHODS

Race differentials: Race differential trials were conducted using six cultivars (Table 1). Trials consisted of three treatments (two NT *Fon* isolates plus an agar control) each with six pots of ten plants. Isolations were made from symptomatic plants, and one single-spore culture was obtained from each pot.

Molecular characterisation: Mycelium was scraped from cultures grown on PDA plates, and DNA was extracted using the DNeasy Plant kit (Qiagen, Australia). All cultures were subjected to the *Fon*-specific PCR test. Putative SIX genes were targeted using published methods (5) and primers designed in-laboratory.

RESULTS AND DISCUSSION

Race differentials: All isolates used in this study were pathogenic to watermelon. First year data from the race differential trials conducted in the NT indicated that the NT isolates were likely to be race 2 (Table 1).

Table 1. Race differential trials conducted in the NT.

Fon*	SB	CS	ChG	AS	CG	SP-4
36953	S	S	R	S	S	R
36955	S	S	R	S	S	R
Race 0	S	R	R	R	R	R
Race 1	S	S	S	R	R	R
Race 2	S	S	S	S	S	R
Race 3	S	S	S	S	S	S

SB- Sugarbaby, CS- Crimson Sweet, ChG- Charleston Gray, CG- Calhoun Gray, AS- Allsweet. **Fon* NT isolate NTP-Dc

The resistance reaction of Charleston Gray did not correlate with expected published data (3) or data obtained for the other cultivars. These trials are currently being repeated.

Molecular characterisation: Each isolate was subjected to the specific *Fon* PCR test and all were positive except for two isolates from USA and two from Australia (Table 2). All *Fon* races were positive for the presence of SIX 8. No correlation of the races and the SIX genes were observed. Bioinformatics also did not show significant differences in the sequences (data not shown). Further molecular characterisation is underway to conduct next generation sequence data for diagnostic markers to distinguish between *Fon* races.

Table 2. Molecular characterisation of *Fon* isolates.

R*	Sample/ Site	<i>Fon</i>	Six genes					
			4	6	8	9	11	13
0	F-121-2/ USA	-	-	+	+	-	-	-
1	F-016-1/ USA	+	-	-	+	+	+	+
1	F-079-1/ USA	+	+	+	+	+	+	+
1	F-107-1/ USA	-	-	-	+	-	+	+
2	F-17B-1-29/ USA	+	+	+	+	+	+	+
2	F-17B-1-3/ USA	+	+	-	+	+	+	+
2	VP 088/ QLD	-	-	-	+	-	+	+
3	MDZE-6221A/ USA	+	-	-	+	+	+	+
3	VP 0457/ NSW	-	-	-	+	-	+	+
3	VP 0583/ WA	+	+	+	+	+	+	+
3	VP 0585/ QLD	+	+	+	+	+	+	+
2	36953/ NT	+	+	+	+	+	+	+
2	36955/ NT	+	+	+	+	+	+	+

*Based upon race differential studies. R= race

ACKNOWLEDGEMENTS

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POSTER 58

Pigments in rust fungi: biosynthesis, role in plant-pathogen interactions, and evolution

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INTRODUCTION

Cereal grains are grown in greater quantities and provide more food energy worldwide than any other crop, and the rust diseases of these crops are among the most feared of all plant diseases. With projected increases of the world's population, there is urgency in finding such new ways to control plant disease.

As long ago as early Greek and Roman civilisations (ca. 500 BC), the feature that epitomised the rust fungi is the distinctive rusty colour of one or more of its spore stages. These distinctive pigments are produced by all of the ca. 7,800 rust species, and are therefore of great biological interest both as a potential generic target in developing new strategies for rust control, and in diagnostics.

It has been demonstrated that one dominant gene in *Puccinia graminis* f. sp. *tritici* (Pgt) controls brown pigmentation in the spore wall and a second, orange carotenoid pigmentation in the cytoplasm (1). Yellow mutants lacked spore wall pigmentation, grey-brown mutants lacked cytoplasmic pigmentation, and albino mutants lacked both.

This project aims to provide a new level of understanding of rust pigments: how they are synthesised in rust spores; variation in pigments both within and between rust species; and what function(s) they serve. The experimental approach will combine analytical chemistry and molecular biology, and will draw on unique resources for cereal attacking rust pathogens. The results will further our understanding of the evolution of the cereal rusts.

MATERIALS AND METHODS

Identification of pigments in rust. A large range of rust species and colour mutants will be analysed for their complement of pigments. Cytoplasmic pigments will be extracted using standard methods (2) that involve urediniospore disruption followed by the use of solvents, drying and filtering, and re-dissolution in acetone. Cell wall localised pigments will be extracted using a melanin extraction procedure (3) whereby spores are boiled in 1.0 M KOH solution for 2 h, cooled and centrifuged. Both cytoplasmic and cell wall pigments will be analysed by reversed-phase HPLC, with identification using a combination of standards and mass spectrometry.

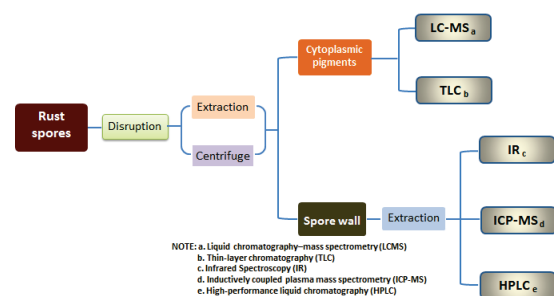


Figure 1. Identification and determination of relative abundance of pigments

Functional annotation of genes involved in *P. hordei* urediniospore pigmentation and elucidation of the pigmentation synthesis pathway. The *P. hordei* genome sequence will be searched using sequences of carotenoid biosynthetic enzymes from fungi, bacteria and plants as queries to identify candidate genes. Complementation studies will be conducted using *Escherichia coli*, a non-carotenogenic bacterium that has been used in previous studies where carotene synthesis genes have been cloned (4).

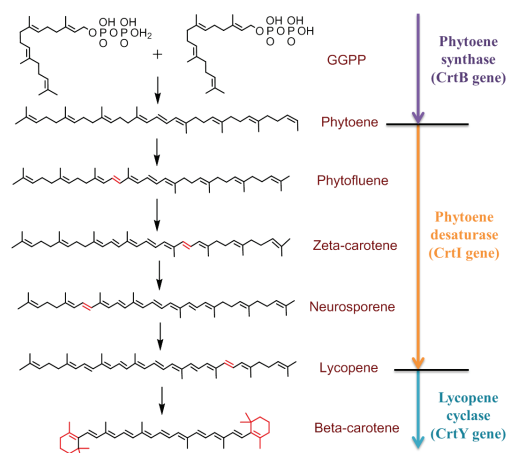


Figure 2. Fungal carotenoid biosynthesis pathway

Testing of pigments function in rust spores. Rust spore germination experiments will be performed on agar in which spores are exposed to a range of doses of UV-B radiation (280 to 315 nm). Comparisons will be made between wild-type and yellow and albino mutants. Oxidative stress will be induced using treatments with hydrogen peroxide (H_2O_2).

ACKNOWLEDGEMENTS



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POSTER 59

Host range of *Neocosmospora* species causing root rot of legumes in AustraliaK.M. Wenham^A, V.J. Galea^A, M.J. Ryley^B, G.C. Wright^C, W. Bryden^A^A School of Agriculture and Food Science, The University of Queensland, Gatton, Queensland, 4343, Australia^B Centre for Crop Health, University of Southern Queensland, Toowoomba, Queensland, 4350, Australia^C Peanut Company of Australia, PO Box 26, Kingaroy, Queensland, 4610, Australia

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INTRODUCTION

Neocosmospora species are the suspected causal pathogens of the emerging soilborne disease *Neocosmospora* root rot, which has been reported in peanut (*Arachis hypogea*) in Australia. The disease has been reported in most peanut growing areas in Australia and has caused an estimated economic loss \$5.4 million annually.

The disease is commonly characterised by wilt-like symptoms and yellowing of the main stem followed by severe root and crown rot and rapid plant necrosis. *Neocosmospora* sp. has had sporadic reports as a pathogen of other leguminous crops such as chickpea (*Cicer arietinum*) and soybean (*Glycine max*) both in Australia and overseas.

Limited research has been conducted and published overseas on the two *Neocosmospora* species thought to be responsible for the disease outbreaks, *F. neocosmosporiellum* and *Neocosmospora striata*, however evidence suggests that the pathogen can invade and survive saprophytically in plant residues of other leguminous species as well as non-leguminous species including cotton (*Gossypium hirsutum*) and sorghum (*Sorghum bicolor*). It is likely that infected residues of these crops contribute to the survival of the pathogen and the build-up of inocula leading to disease epidemics when conditions are conducive

MATERIALS AND METHODS

Commercial cultivars of peanut, chickpea, soybean, mung bean, sorghum, wheat, maize and cotton were used for this study based on historical crop rotation data from peanut growing regions in Australia.

Seed of each species was surface sterilised and sown on 2% water agar to germinate for seven days. Five seeds from each species were transplanted into pasteurised potting mix 150mm plastic pots until five replications were planted for each crop species.

Plants were thinned to one plant per pot after establishment and watered as required until 30 days after planting.

At 30DAP each plant per replication was assigned a treatment. Treatments included root inoculation, soil infestation, stem wounding with a mycelium plug, mycelium plug with no stem wound and control plants not subjected to inoculation.

Plants were maintained on the glasshouse bench at 21-30°C±2° for eight weeks and watered as required before 5mm sections of plant tissue were placed onto half strength potato dextrose agar amended with 0.01% streptomycin sulfate (1/2PDAS) and incubated at 25°C for 14 days.

RESULTS

Eight weeks post-inoculation all crop species from each replication were harvested and assayed for signs of infection by *Neocosmospora* sp. None of the plants

exhibited typical field symptoms of *Neocosmospora* root rot infection such as chlorosis or wilting. All plants subjected to stem inoculation with mycelium plugs treatments developed clear lesions around the site of inoculation and *Neocosmospora* sp. was re-isolated from vascular tissue 10mm from the edge of the internal stem lesion for all plant species. Stem wounding resulted in a higher % of colonised plant species (Figure 1) however the fungus was still able to colonise plants without a wound to act as an entry point for infection for peanut, chickpea, soybean and cotton plants. The root inoculation and soil infestation treatments did not exhibit any symptoms of infection. *Neocosmospora* sp. was re-isolated from the peanut plant however did not colonise any other plant species.



Figure 1. The percentage of total plants colonised by *Neocosmospora* sp. through stem inoculations.

DISCUSSION

Preliminary investigations indicate *Neocosmospora* sp. are able to colonise a wide plant host range as endophytes. This is likely to be the major contributor to the build-up of soil inocula, leading to disease outbreaks in susceptible plant species due to the survival of the fungus in plant residues post-harvest.

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POSTER 60

Are Western Australia's horticultural crops susceptible to southern sting nematode?

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INTRODUCTION

Southern sting nematode (*Ibipora lolii*) is a major pest in Western Australian (WA) turf farms, sporting fields, amenities grounds and golf courses. It is thought that it was introduced into WA in the 1970s on turf from New South Wales. They are large ectoparasites (2-3mm long) feeding primarily on root tips causing stubby roots that do not function efficiently. Once turf is infested, eradication is not possible so management is required to minimise symptoms.

In WA, concerns are held that southern sting may infest horticultural crops as land used for turf production is also ideal for vegetable production. A sting nematode with similar morphology (*Belonolaimus longicaudatus*), found in the USA, is a damaging pest of both turf grasses and vegetable crops. *Belonolaimus* prefers irrigated coastal sandy soils similar to WA's horticultural area on the swan coastal plain.

The aim of this glasshouse trial was to determine if vegetable crops commonly grown on Perth's swan coastal plain are as susceptible to the southern sting nematodes as a known host, kikuyu.

MATERIALS AND METHODS

Methods for determining the ability of *I. lolii* to multiply were adapted from Stirling *et al*, 2013. Lettuce (green oak, Kiribati), onion (Mercedes), carrot (Stefano), broccoli (Bridge), tomato (Swanson), potato (Nadine), English spinach (Tapir) and kikuyu seeds were planted into 2000 cm³ pots filled with pasteurised soil from Medina Research Station (soil representative of Perth's swan coastal plain horticultural area). The trial was in a heated glasshouse with an average soil temperature of 21°C (average daily max 30°C, min 18°C).

For inoculum soil was sourced from an infested turf farm and sting nematodes were extracted using Whitehead trays. Two weeks after seeding, pots were inoculated with approximately 20 nematodes/200 mL soil. For each plant type, five inoculated and two un-inoculated pots were arranged in a randomised block design. At 5 and 10 weeks after inoculation five soil cores (ca 500 g dw soil) and at 15 weeks, 10 cores (ca 920 g dry weight soil) were taken from each pot. Nematodes were extracted on Whitehead trays for five days and the number of *I. lolii*/pot was calculated. At the final sampling, soil was washed off the roots and dry root and top weights were recorded.

RESULTS

Kikuyu and broccoli were the only crops where sting nematodes were detected at more than one sampling time (Figure 1). At the final harvest *I. lolii* was detected in all inoculated kikuyu pots with an average of 1600 nematodes/pot. It was also detected in three of five pots of inoculated broccoli with an average of 93 nematodes/pot. The approximate multiplication rate in kikuyu was 8 and in broccoli 0.5.

Inoculation with *I. lolii* caused a significant decrease in dried shoot biomass of Bridge broccoli ($p=0.04$). Inoculation of kikuyu grass significantly decreased root biomass compared to non-inoculated plants ($p=0.002$). No other crops were significantly affected.

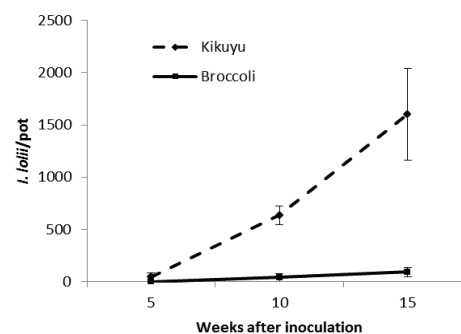


Figure 1. Number of *Ibipora lolii* in soil of pots containing kikuyu and broccoli plants after inoculation with 197 nematodes/pot in a glasshouse trial ($n=5$).

DISCUSSION

Kikuyu, the positive control, was the most susceptible plant tested. Broccoli was the only horticultural crop from which *I. lolii* was detected in more than one rep over more than one sampling time. Whilst broccoli supported sting nematodes, the population did not increase as rapidly as it did in the susceptible control, kikuyu. However, infestation with *I. lolii* resulted in 22% reduction in the above ground biomass of broccoli, indicating the nematodes were affecting plant growth even though the population was low. Reduced top weight may affect yield which would be of concern to growers.

Although testing only a limited number of crop varieties, the results indicate that southern sting nematode may not be a threat to many of the commonly grown horticultural crops in WA, with turf grasses the most susceptible hosts. For other plant parasitic nematodes there can be a large variation in resistance between varieties of a particular crop. Therefore testing more varieties of horticultural crops is necessary to confirm this result. Grass crops such as wheat and barley, may be more susceptible than vegetables. Stirling *et al* (2013) found sugar cane was as susceptible to *I. lolii* as kikuyu. The potential impact of southern sting nematode on broad acre crops should also be determined.

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POSTER 61

Managing *Aphanomyces trifolii*, a cause of severe root disease in high rainfall subterranean clover pastures

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INTRODUCTION

Aphanomyces euteiches was reported as widespread on diseased roots of subterranean clover (*Trifolium subterraneum*) pastures across south-eastern Australia (1). However, now it is clear that those isolates of *Aphanomyces* from diseased subterranean clover roots in eastern Australia had been mistakenly labelled as *A. euteiches*, and were in fact a new species, *A. trifolii* (2).

MATERIALS AND METHODS

Thirty-eight subterranean clover varieties were tested against a mixture of 20 *A. trifolii* isolates collected in 2013, mixed at equal volume proportions. Tap and lateral root disease were computed as a % index (root rot disease index = sum of all root disease ratings × 100/total number of plants × maximum rating value) for each subterranean clover variety. There were six replications each for inoculated and non-inoculated pots for each variety in a completely randomised design.

RESULTS AND DISCUSSION

All tested varieties had PDI > 20 for both tap and lateral root disease. The most resistant varieties in terms of both tap and lateral root disease to the mixed isolates of *A. trifolii* were Antas, Uniwager and Leura, with PDI < 33 for tap roots and < 48 for lateral roots. The least resistant varieties were Mt. Barker, York and Woogenellup, with PDI > 50 for tap roots and > 70 for lateral roots. There was a strong correlation between tap and lateral root disease. This is the first resistance to *A. trifolii* identified and these varieties will perform well under field conditions conducive to *A. trifolii*. They offer new opportunities to both manage this important root pathogen using current resistant varieties and for breeding of new resistant varieties.

Table 1. The effects of a mixture of 20 isolates of *Aphanomyces trifolii* on tap and lateral root % disease indices for tested subterranean clover varieties.

Variety	Tap DI%	Lateral DI%
Antas	21	47
Bacchus Marsh	34	49
Campeda	35	59
Clare	29	60
Dalkeith	44	65
Dinninup	37	44
Dwalganup	44	52
Enfield	37	47
Esperance	37	57
Geraldton	51	63
Gosse	26	47
Goulburn	48	64.
Green Range	44	62.
Howard	45.	71
Izmir	56	69
June	46	69
Karridale	36	61
Larisa	45	63
Leura	33	48
Losa	45	50
Meteora	41	73
Mintaro	38	62
Mt Barker	58	76
Nangeela	54	70
Napier	31	52
Narrikup	49	71
Northam	26	42
Nuba	44	66
Nungarin	43	43
Riverina	36	66
Rosedale	40	60
Seaton Park	45	49
Tallarook	49	67
Trikkala	45	77
Uniwager	31	30
Urana	22	35
Woogenellup	50	70
Yarloop	50	71
York	56	74
<i>l.s.d. (P<0.05) =</i>	<i>13.8</i>	<i>14.2</i>

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POSTER 62

Suppressive effect of vermi-composted bamboo-powder on *Pythium ultimum* var. *ultimum*X.D. You^A, Y. Shimogami^B, A. Matsumura^A and M. Tojo^A^A Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai City, Osaka 599 8531 JAPAN^B Environmental Policy Division, Kawachinagano City Office, Kawachinagano City, Osaka 586 8501 JAPAN

INTRODUCTION

Bamboo is prevailing near populated areas in Japan and cause significant impacts on diversity, terrestrial water system and carbon cycle in the ecosystem. Since bamboo contains diverse organic and inorganic nutrients, its converting into composts is a promising usage. The use of earthworms for composting organic biosolids, termed 'vermicomposting', is a sustainable- and cost-effective approach for the management of biodegradable solid wastes. Commercial paper and food waste vermicomposts can suppress soil borne pathogens such as *Pythium*, *Rhizoctonia solani*, *Verticillium* (1). However, no information is available on the feasibility of bamboo vermicomposting and its suppressive effects against soil borne pathogens.

The objectives of this study were to evaluate the ability of earthworm to degrade bamboo into vegetable growth medium and to demonstrate the disease suppressiveness of the medium infested with a soil borne pathogen *P. ultimum* var. *ultimum*.

MATERIALS AND METHODS

Preparation of vermi-composted bamboo-powder. One-year-old shoots of moso bamboo (*Phyllostachys edulis* (Carrière) J. Houz.) were ground into powder using a grinder, and soaked in ten times volume of tap water for 24 hours to remove detrimental compounds such as saponin. Ten kg of the powder was mixed with 100 g of earthworm (*Eisenia fetida* Savigny), 100 g of dried powder of kudzu (*Pueraria lobata* (Willd) Ohwi.) as a nitrogen source, and 20 g of commercial horse manure/wheat straw compost (JRA Facilities, Tokyo, Japan) as a soil-microbial source. The mixture was placed in a plastic box, covered with a lid, incubated at 28±2°C for two months, and watered every other day to keep moisture at approximately 80%. Fertility properties (Inorganic N, PO₄³⁻ and K⁺) of the mixture were measured by using pack tests (Kyoritsu Chemical-check Lab Co, Tokyo, Japan). A commercial nursery soil (Aisai-1, Katakura Chikkarin, Tokyo, Japan) was used as control. There were five replicates for each treatment.

Evaluation of disease suppressiveness. Six-day-old seedlings of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*, cv. Nanami) and cucumber (*Cucumis sativus* L., cv. Aonagakei-jibai), and 12-day-old seedlings of spinach (*Spinacia oleracea* L., cv. Jiromaru) grown in the commercial nursery soil were used. Inoculum of *Pythium ultimum* var. *ultimum* isolate MAFF240023 was prepared by the bentgrass-seed culture method (2). One g of the inoculum was thoroughly mixed with 100 g of the compost in a mortar and placed in a plastic pot. The seedlings were transplanted into the infested compost and grown in an incubator at 25°C (night 12h) / 28°C (day 12h) with continuous light and irrigated daily with tap water. Mortality of the seedlings was determined 7 days after transplanting. Five pots were used for each host plant. Each pot had 5 seedlings.

RESULTS AND DISCUSSION

The vermi-composted bamboo-powder had equivalent or higher inorganic N, PO₄³⁻ and K⁺ as compared to the commercial nursery soil (Table 1). The compost significantly suppressed damping-off of cucumber, Chinese cabbage and spinach (Table 2).

The equivalent or higher NPK content of the compost and its suppressiveness against *P. ultimum* var. *ultimum* indicate that the vermi-composted bamboo-powder has the agronomic potential as a vegetable growth medium.

Table 1. Fertility properties of vermi-composted bamboo - powder (VBP) and a commercial nursery soil (CNS)

	Inorganic N (mg/kg)	PO ₄ ³⁻ (mg/kg)	K ⁺ (mg/kg)
VBP	440±14.7	183±48.0	752±137.0
CNS	594±19.0	240±27.7	55±1.4

Data are mean ± standard error (N = 5).

Table 2. Effect of vermi-composted bamboo-powder (VBP) and a commercial nursery soil (CNS) on vegetable damping-off caused by *Pythium ultimum* var. *ultimum* (PU).

	Mortality (%)		
	Cucumber	Spinach	Chinese cabbage
VBP+PU	0	8±4.4	0
VBP	0	0	0
CNS+PU	44.0±14.3*	80.0±5.7*	28.0±9.1*
CNS	0	0	0

Data are mean ± standard error (N = 5). Asterisk in the same column shows significant difference according the Tukey-Kramer honestly significant difference analysis (P < 0.05).

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POSTER 63

Study on contamination of canola seeds in plants infected to blackleg and sclerotinia stem rot disease

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INTRODUCTION

Oil seeds are very important in our country. Among them canola is more important from the acreages and yield.

Diseases of canola including stem white rot caused by *Sclerotinia Sclerotiorum* and blackleg caused by *phoma lingam* are considered as the most important diseases in Iran. Seed infections have their own importance and vary annually under different conditions.

MATERIAL AND METHODS

At the current investigation, 26 canola fields located in three areas eastern (Kalaleh), central (Aliabad) and Western (Kord kouy) of Golestan province were selected to study both diseases. At the cropping season, seed samples of every 10 plants demonstrated the diseases were collected separately and transformed to plant pathology laboratory. Seed weight and seed germination factors were measured for each sample even non-infected ones. Employing fungal identification keys, the colonies grown were studied.

RESULTS AND DISCUSSION

The seed weight of samples infected to blackleg did not show considerable difference with control samples whereas *Sclerotinia* had decreased this factor totally. Also there were not sensible differences between treatments for seed germination. Fungi including *Alternaria*, *Penicillium*, *Aspergillus* infected plants to blackleg did not result in seed contamination and only one sample of *Sclerotinia* affected plants demonstrated the fungal colonies of the pathogen.

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POSTER 64

High incidence of cereal viruses in Victorian crops

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INTRODUCTION

Barley yellow dwarf virus (BYDV) and *Cereal yellow dwarf virus* (CYDV) are the two most wide spread and economically important viruses of cereal crops worldwide, reducing yield and quality. Despite their importance, little information is available on their distribution in the cereal growing regions of Victoria, Australia. To address this knowledge gap, a survey was done in the Western and Southern Victoria in 2014.

MATERIALS AND METHODS

Two thousand random cereal plants were collected across 20 cereal crops (12 wheat and 8 barley) and tested using tissue blot immunoassay (TBIA) and reverse-transcription polymerase chain reaction (RT-PCR) to determine the incidence of BYDV and CYDV in the field.

The presence of CYDV, BYDV-PAV and BYDV-MAV were determined using TBIA and RT-PCR, whilst BYDV-SGV and BYDV-RMV were only tested by RT-PCR (Table 1).

Table 1. Primers, targeted species/strains, annealing temperature (TM), product size and references of the 9 RT-PCR tests used to detect BYDV and CYDV

Primer name	Target species / strains	Tm (°C)	Product size (bp)	Reference
CYDV-1	CYDV	60°C	719	Rastgou et al 2005 [1]
CYDV-2				
RPV R	CYDV	55°C	400	Deb & Anderson 2008 [2]
RPV L				
PAV F	PAV	52°C	520	Nagy et al 2007 [3]
PAV R				
BYDV-1	PAV	60°C	744	Rastgou et al 2005 [1]
BYDV-2				
PAV L1	PAV	55°C	295	Deb & Anderson 2008 [2]
PAV R1				
PAV CpF	PAV	55°C	91	[4]
PAV CpR				
MAV L1	MAV	55°C	175	Deb & Anderson 2008 [2]
MAV R1				
SGV L2	SGV	52°C	237	Deb & Anderson 2008 [2]
SGV R2				
RMV L1	RMV	59°C	365	Deb & Anderson 2008 [2]
RMV R				

RESULTS

The within crop incidence of BYDV-PAV, BYDV-MAV and CYDV ranged from 3-58%, 0-39% and 1-17% respectively as determined by TBIA and the incidence of BYDV was higher in wheat compared with barley crops.

Based on the RT-PCR results, almost 80% of all samples tested were positive for either BYDV and/or CYDV (Figure 1). Most of the infected crops were found to be co-infected with different strains of BYDV and/or CYDV. The number of plants that tested positive for virus infection were dependent on the diagnostic assays used and a sample could be positive in only one or several of the four BYDV-PAV RT-PCR tests.

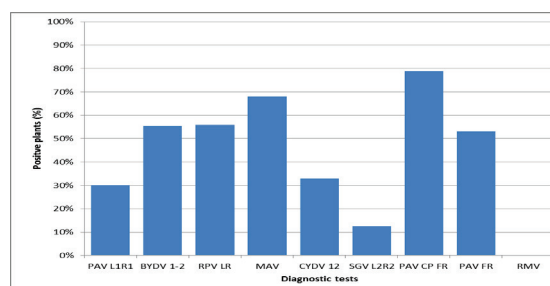


Figure 1. Percentage of pooled samples tested positive for either BYDV and/or CYDV using 9 different diagnostic tests

DISCUSSION

Our results indicate that BYDV and CYDV are widely distributed in the cereal growing regions of Victoria and management strategies are needed to keep these viruses under control. Results from the RT-PCR tests suggest that genetic variations exist in the BYDV and CYDV population. It also highlights an urgent need for the consolidation of diagnostic tests for the detection of BYDV and CYDV.

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