APPS 2009

Plant Health Management: An Integrated Approach

29 September – 1 October 2009
Newcastle City Hall

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Contents

Welcome

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

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

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

David Guest
Conference Convenor, APPS 2009

Conference Organising Committee

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

Workshop Convenors

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

Tree Pathology Workshop
—André Drenth and Angus Carnegie

Magical Mystery Vegetable Tour
—Len Tesoriero and Nerida Donovan

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

Conference Secretariat

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CONFERENCE LOGISTICS

Wednesday 30 September

0800–1730	Registration open	Concert Hall Foyer
0800	ARRIVAL TEA AND COFFEE	Concert Hall Foyer
0830	Keynote address—*Molecular cytology of Phytophthora-plant interactions* **Prof Adrienne Hardham**, Plant Cell Biology Group, Research School of Biology, The Australian National University	Concert Hall

	Session 4A **Plant pathogen interactions** **Room:** Concert Hall **Chair:** David Guest	**Session 4B** **Disease surveys** **Room:** Cummings Room **Chair:** Sandra Savocchia	**Session 4C** **Epidemiology** **Room:** Hunter Room **Chair:** Greg Johnson	**Session 4D** **Prokaryotic pathogens** **Room:** Newcastle Room **Chair:** Lucy Tran-Nguyen
0910	*Gene expression changes during host-pathogen interaction between Arabidopsis thaliana and Plasmodiophora brassicae* **Mrs Arati Agarwal**, Department of Primary Industries, Vic	*Prevalence and pathogenicity of Botryosphaeria lutea isolated from grapevine nursery materials in New Zealand* **Ms Regina Billones**, Lincoln University, NZ	*Honey bees— do they aid the dispersal of Alternaria radicina in carrot seed crops?* **Mr Rajan Trivedi**, Lincoln University, NZ	*Transmission of 'Candidatus Phytoplasma australiense' to Cordyline and Coprosma* **Dr Ross Beever**, Landcare Research, NZ
0930	*Hairpin RNA derived from viral NIa gene confers immunity to wheat streak mosaic virus infection in transgenic wheat plants* **Mr Muhammad Fahim**, CSIRO Plant Industry, and Australian National University, ACT	*Infection and disease progression of Neofusicoccum luteum in grapevine plants* **Mr Nicholas Amponsah**, Lincoln University, NZ	*Translating research into the field: meta-analysis of field pea blackspot severity and yield loss to extend model application for disease management in Western Australia* **Dr Moin Salam**, Department of Agriculture and Food WA	*Australian grapevine yellows phytoplasma found in symptomless shoot tips after a heat wave in South Australia* **Mr Peter Magarey**, South Australian Research and Development Institute, SA
0950	*Characterising inositol signalling pathways in Phytophthora spp. for future development of selective antibiotics* **Mr Dean Phillips**, Deakin University, Vic	*Carbohydrate stress increases susceptibility of grapevines to Cylindrocarpon black foot disease* **Miss Dalin Dore**, Lincoln University, NZ	*Development of a model to predict spread of exotic wind and rain borne fungal pests* **Dr Moin Salam**, Department of Agriculture and Food WA	*Association of Phytoplasmas with papaya crown yellows (PCY) disease—a new disease of papaya in Northern Mindanao, Philippines* **Ms Regina Billones**, Del Monte Phils Inc, Philippines
1010	*Systemic acquired resistance—a new addition to the IPM clubroot toolbox?* **Dr Caroline Donald**, Department of Primary Industries, Vic	*Botryosphaeria spp. associated with bunch rot of grapevines in south-eastern Australia* **Ms Nicola Wunderlich**, Charles Sturt University, NSW	*Psyllid transmission of Huanglongbing from naturally infected Shogun mandarin to orange jasmine* **Dr Rantana Sdoodee**, Prince of Songkla University, Thailand	*Phytoplasma diseases in citrus orchards of Pakistan* **Dr Shazia Mannan**, COMSATS Institute of Information Technology, Pakistan
1030	MORNING TEA			Banquet Room
1100	Keynote address—*Mechanisms modulating fungal attack in postharvest pathogen interactions and their modulation for improved disease control* **Prof Dov Prusky**, Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, Israel			Concert Hall

	Session 5A **Plant pathogen interactions** **Room:** Concert Hall **Chair:** Rosalie Daniel	**Session 5B** **Disease surveys** **Room:** Cummings Room **Chair:** Aaron Maxwell	**Session 5C** **Chemical control** **Room:** Hunter Room **Chair:** Len Tesoriero
1140	*ABA-dependant signalling of PR genes and potential involvement in the defence of lentil to Ascochyta lentis* **Dr Rebecca Ford**, The University of Melbourne, Vic	*Fishing For Phytophthora across Western Australia's water bodies* **Dr Daniel Hüberli**, Murdoch University, WA	*Evaluation of fungicides to manage brassica stem canker* **Ms Lynette Deland**, South Australian Research and Development Institute, SA
1200	*Fundamental components of resistance to Phytophthora cinnamomi: using model system approaches* **Prof David Cahill**, Deakin University, Vic	*Incidence of fungi isolated from grape trunks in New Zealand vineyards* **Mr Dion Mundy**, The New Zealand Institute for Plant and Food Research Limited, NZ	*Evaluation of spray programs for powdery mildew management in greenhouse cucumbers* **Dr Kaye Ferguson**, South Australian Research and Development Institute, SA
1220	*Genes involved in hypersensitive cell death responses during Fusarium crown rot infection in wheat* **Dr Jill Petrisko**, University of Southern Queensland, Qld	*Isolation and characterisation of strains of Pseudomonas syringae from waterways of the Central North Island of New Zealand* **Dr Joel Vanneste**, The New Zealand Institute for Plant and Food Research Limited, NZ	*The incidence of copper resistant bacteria in Australian pome and stone fruit orchards* **Dr Chin Gouk**, Department of Primary Industries, Vic

Thursday 1 October

0700	Regional Councillor's meeting	Waratah Room
0700	CHAIRMAN'S BREAKFAST	Mulumbinba Room
0800–1730	Registration open	Concert Hall Foyer
0800	ARRIVAL TEA AND COFFEE	Concert Hall Foyer
0830	Keynote address—*Translating research into the field: how it started, how it is practised and how we carry out grape powdery mildew research* **Dr Bob Seem**, Cornell University, USA	Concert Hall
0910	GRDC book launch: **Mr James Clarke**, Grains Research and Development Corporation	Concert Hall

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

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

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INTRODUCTION

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

MATERIALS AND METHODS

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

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

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

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

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

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

RESULTS AND DISCUSSION

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

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

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

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

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4. Mergoum M, Hill JP, Quick J (1998). Evaluation of resistance of winter wheat to *Fusarium accuminatum* by inoculation of seedling roots with single, germinated macroconidia. *Plant Dis* **82**, 300–2.
5. Desmond OJ, Manners JM, Stephens AE, Maclean DJ, Schenk PM, Gardiner DM, Munn AL, Kazan K (2008) The Fusarium mycotoxin deoxynivalenol elicits hydrogen peroxide production, programmed cell death, and defence responses in wheat. *Mol Plant Pathol* **9**, 435–45

Symptom development and pathogen spread in wheat genotypes with varying levels of crown rot resistance

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INTRODUCTION

Crown rot, caused by *Fusarium pseudograminearum (Fpg),* is an important soilborne disease of winter cereals. Complete resistance has yet to be reported in any wheat genotypes and hence is an ongoing issue for Australian wheat growers. In order to understand the nature of the partial resistance identified to crown rot we have examined the patterns of disease and pathogen spread in both susceptible and partially resistant tissues. Field trials were designed to study disease symptom development and localisation of *Fpg* hyphae in the bread wheat varieties Puseas, Vasco and Sunco, and the line 2–49.

MATERIALS AND METHODS

Inoculated field trials were conducted, using a randomised block design. Inoculum was placed in a band lying above the seed at sowing. Five plants from three replicates were harvested at approximately fortnightly intervals throughout the growing season. Leaf sheaths and internodes of the 1st 5 tillers were rated for disease using a scale from 0 to 4 as described in Wildermuth & McNamara (1), where 0 = no lesions evident and 4 = >75% of tissue lesioned. Following disease rating, each tissue piece from two replicates was surface sterilised and plated out on Czapek Dox agar. Plates were checked daily for 5 days after plating. Sites of colony emergence were marked with ink on the abaxial plate surface.

Disease rating data were analysed untransformed and the isolation counts were square-root transformed prior to analysis. Restricted Maximum Likelihood (REML) Variance Components Analysis was used to determine significance of the fixed factors harvest, genotype, tiller and the corresponding two and three way interactions. To determine where individual means were significantly different 95% confidence intervals of error were calculated for each analysed plant part.

RESULTS AND DISCUSSION

Differences in moisture conditions between the two field trials resulted in differences in overall plant development, extent of *Fpg* colonisation and symptom expression. The results of the second trial conducted under higher moisture conditions will be presented here.

Disease symptoms developed and *Fpg* was isolated from plant parts of all tillers of all genotypes. Statistically significant differences between genotypes were not expressed in the disease rating or isolation of *Fpg* from leaf sheath tissue in field trials even at the seedling stage (data not shown). Significant differences were seen between partially resistant and susceptible wheat genotypes in both disease rating and isolations from internode tissues and this could be detected soon after stem extension commenced (Figures 1 and 2).

Large differences in symptom expression were seen between genotypes in internode 1 around anthesis but not at maturity. This is an important observation as maturity is a favoured time for rating field material for crown rot screening. At later harvests differences between genotypes were clearly expressed in higher internodes and at maturity lesions had developed as high as the 2nd internode in 2–49, 4th in Sunco, and the 5th internode in Puseas and Vasco. At maturity *Fpg* was consistently recovered from the 4th internode in 2–49 and the 5th in all other tested genotypes, indicating a delay in symptom expression in the infected 2–49 tissues.

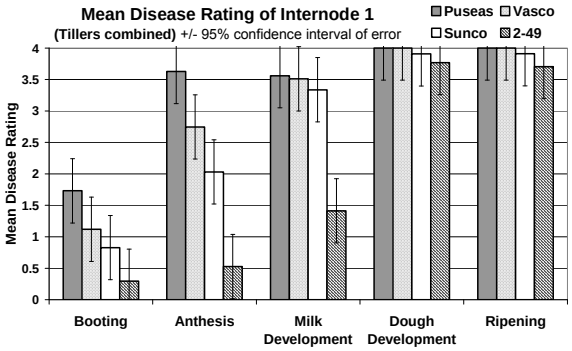

Figure 1. Mean disease rating of internode 1 at 13 (booting) to 22 weeks after planting (WAP) (ripening). n = 75 tillers

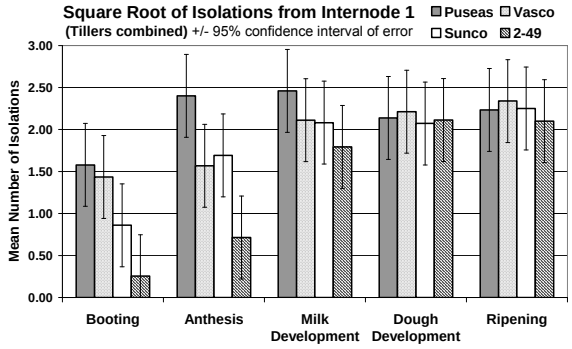

Figure 2. Mean isolations from internode 1 at 13 (booting) to 22 WAP (ripening). n = 50 tillers

CONCLUSIONS

Resistance was expressed as a slowing down of colonisation of plant parts in 2–49 and to a lesser extent in Sunco when compared to the susceptible genotype Puseas. Both colonisation and disease symptoms are initially slowed in young tissues of partially resistant genotypes however at later harvest times these same tissues may be as infected and symptomatic as the tissues of susceptible genotypes.

ACKNOWLEDGEMENTS

Financial support provided by GRDC PhD scholarship to CDP.

REFERENCES

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Crown rot of winter cereals: integrating molecular studies and germplasm improvement

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INTRODUCTION

Crown rot of winter cereals is a major constraint on grain production across most growing regions in Australia, particularly where stubble retention is practiced to maintain soil structure and retain soil water. The predominant cause of this disease is infection with *Fusarium pseudograminearum* (*Fpg*), although in some southern areas *Fusarium culmorum* infections are also significant. These Fusarium species are able to grow saprophytically on stubble remnants over the summer and provide inoculum for crop infection in the following season. Losses due to crown rot are highest in seasons featuring a dry finish in which maturing plants experience water stress, with symptoms including basal stem browning and white heads bearing no grain.

Control of this disease is challenging and is currently based on management practices centred on crop rotation strategies. At present, there are no resistant commercial varieties of bread wheat, durum or barley available for deployment. Durum wheats are particularly susceptible.

We are currently undertaking a long-term collaborative research program which aims to:

- characterise known resistance sources
- develop molecular markers for quantitative trait loci (QTL) to assist selection in breeding programs
- transfer QTL for resistance from hexaploid (bread) to tetraploid (durum) wheats
- pyramid resistance in bread wheats
- understand the fundamental biology of this host/pathogen interaction.

Central to the task is an integration of laboratory and field-based investigations to ensure outcomes that not only advance our knowledge but also reduce yield losses and increase management options for primary producers.

Here we report on recent successes in pyramiding sources of partial resistance and discuss progress in transferring resistance from hexaploid sources into a durum background.

METHODOLOGY

Two doubled haploid wheat populations produced from crosses of partially resistant parents, Sunco/2-49 and 2-49/W21MMT70 were evaluated for resistance to crown rot using a standard seedling pot test inoculated with a mixture of aggressive *Fpg* isolates(1). Based on genetic maps constructed from SSR and DArT markers, QTL for resistance were then identified.

Crosses between hexaploid wheat lines with partial resistance and a range of durum lines were obtained from Dr Ray Hare, NSW DPI. These materials were field grown near Tamworth NSW in *Fpg* infected plots through to the F7 generation and assessed for crown rot susceptibility each season.

RESULTS AND DISCUSSION

To date, a wide selection of resistance sources have been partially characterised and quantitative trait loci (QTL) identified (2, 3, 4). Inoculated seedling and field trials indicate overlapping sets of loci that contribute at these different stages of development. These partial sources of resistance contain largely different sets of QTL which suggest that improved resistance may be obtained by gene pyramiding. Results from QTL analysis of the Sunco/2-49 and 2-49/W21MMT70 populations following seedling trials indicate that the more resistant lines inherited the major QTL from each parent and that a number of lines in the 2-49/W21MMT70 population expressed significantly higher resistance than either of the parents. Hence pyramiding of independent resistance sources can produce significant improvements in the resistance of derived progeny towards crown rot.

Marker analysis of hexaploid x tetraploid crosses shows that the bread wheat markers were readily transferred to the progeny. Furthermore even after several generations these markers remained linked to the resistance character and were independent of remnant D genome material. Crosses of durum wheats with the hexaploid resistance sources significantly reduced the disease severity in derived materials, demonstrating the potential of this approach for improving the resistance of durums to crown rot.

ACKNOWLEDGEMENTS

We acknowledge the contributions of Dr Graham Wildermuth and Dr Ray Hare to this work. This work was funded by the Grains Research and Development Corporation (GRDC).

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Infection of wheat tissues by *Fusarium pseudograminearum*

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INTRODUCTION

Crown rot of wheat, caused by *Fusarium pseudograminearum* (*Fp*), is a serious disease threat across the Australian wheat belt. Currently control of this disease relies on farming practices (e.g. crop rotation) and planting of less susceptible cultivars. Partial resistance has been identified in a small number of wheat lines, such as 2–49 and Sunco, but the mechanisms of resistance shown by these lines have not been identified.

Partial resistance can be expressed in either the seedling or adult stage, depending on the genotype, with the majority of current screening methods being based on seedling scoring. Extensive seedling trial comparisons between susceptible and partially resistant host genotypes suggest a significantly slower spread of the fungus in the younger tissues of resistant individuals (1).

The current project aims to assess growth of *Fp* during crown rot development across partially resistant and susceptible wheat lines in order to determine key elements in the progress of disease and when resistance mechanisms are induced. Current disease rating systems for seedlings rely heavily on browning of leaf sheaths and tiller bases (2). Our current investigations are centred on the relationship between the expression of these visible disease symptoms and the extent of fungal infection. These studies are also comparing the progress of fungal spread in both susceptible and partially resistant wheats. The increase in fungal load in each inoculated host genotype has been measured using a quantitative real time multiplex polymerase chain reaction (PCR) assay, allowing simultaneous detection of both pathogen and host DNA. Microscopy of infected wheat tissues is also in progress.

MATERIALS AND METHODS

Inoculation. Two week old seedlings were inoculated using a 10^6 conidia per mL suspension (3). Seedling tissues (particularly leaf sheaths) were harvested at different time points after infection, with four host genotypes being compared (Table 1).

Table 1. Genotypes tested and their resistance rating from field trials.

Genotypes	Crown Rot Resistance Rating
2-49	Partially Resistant
Wylie	Moderately Susceptible
Gregory	Susceptible
Puseo	Highly Susceptible

DNA Extraction and Multiplex Quantitative PCR. DNA was extracted using the DNeasy Minikit (Qiagen). Primers and probes were designed from Genbank sequences with Primer3 software using the translation elongation factor (TEF)-α sequence of *Fp* and the TEF-G sequence of wheat. PCR results were normalised by expressing *Fp* DNA content relative to the host DNA.

Microscopy. Fixation and clearing of tissues was performed as described in (4). Differential staining used safranin and solophenyl flavine dyes. Viewing of tissue was performed using a fluorescence microscope (Nikon Eclipse) under the UV-2A filter.

RESULTS AND DISCUSSION

A strong relationship was observed between visual rating scores of wheat leaf sheaths and the normalised *Fp* DNA (Fig. 1). This demonstrates that the degree of visual discolouration of wheat leaf sheaths correlates with the quantity of *Fp* mycelium present in the tissue, validating visual rating systems of basal discolouration (2,3) as a relative estimation of tissue infection levels across a seedling trial.

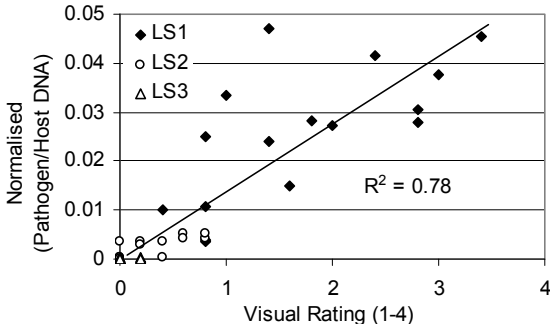

Figure 1. Comparison of levels of normalised *Fp* DNA and visual rating scores of leaf sheaths (LS) 1, 2 and 3 of the four host genotypes at 7 days after inoculation.

Observation of *Fp* growth at increasing periods after inoculation has revealed significant differences in growth of *Fp* in seedlings of the four standard genotypes.

Microscopic assessment of *Fp* growth has observed intra- and inter-cellular growth associated with the leaf sheath epidermis, including trichomes and stomata. Current investigations are examining growth of mycelium in vascular tissues of expanded tillers.

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74 Genetic diversity and population structure of Australian and South African *Pyrenophora teres* isolates

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INTRODUCTION

Net blotch, caused by the fungus *Pyrenophora teres*, is a serious production problem for the barley (*Hordeum vulgare* L.) industry in Australia, South Africa and elsewhere (1, 2, 3, 4). Two forms of net blotch exist: one is the net form (NFNB) caused by *P. teres* f. *teres* (PTT) and the other is the spot form (SFNB) caused by *P. teres* f. *maculata* (PTM). Several Australian and international studies have used molecular markers, such as amplified fragment length polymorphisms (AFLP) to investigate the genetic structure of *P. teres* (3, 5, 6, 7). In contrast, while the incidence of net blotches on barley have increased recently in South Africa, local populations of the fungus have remained uncharacterised. To address this issue, PTT and PTM isolates were collected from the south-western Cape region of South Africa. AFLP analysis was conducted on extracted DNA from these isolates and from a collection of Australian isolates to determine the genetic diversity and structure of South African populations and to determine their relatedness to Australian isolates.

MATERIALS AND METHODS

DNA extractions. Fungal mycelium were harvested from cultures grown on potato dextrose agarose plates at 25°C for one week. A CTAB DNA extraction method was used to extract the fungal DNA.

AFLP analysis. The AFLP procedure was carried out using an Invitrogen AFLP Core Reagent kit. The *EcoR*I primers were hex-labelled. The samples were visualised using a Gel-Scan 2000™ DNA fragment analyser (Corbett Life Sciences, Sydney, Australia).

Scoring and data analysis. Both monomorphic and polymorphic bands were scored and used in the data analysis. Bands were scored independently by two people. The cluster analysis was performed using NTSYSpc V2.20f, whereas the program Structure V2.2 was used to determine the population structure.

RESULTS

AFLP analysis was conducted on DNA of 23 South African and 37 Australian PTT isolates, 37 South African and 29 Australian PTM isolates, six *Bipolaris sorokiniana* isolates, two *P. tritici-repentis* and two *Drechslera rostrata* isolates. Eight primer combinations were used to amplify AFLPs and on average 50 loci were produced with each primer combination. In total, 400 loci could be accurately scored across all samples and 168 of these loci were polymorphic in the *P. teres* samples.

Cluster analysis separated the NFNB and SFNB isolates into two strongly divergent groups (similarity coefficient = 0.6). Low genetic differentiation was observed within the NFNB and SFNB groups (similarity coefficient = 0.9). Interestingly, the South-African NFNB isolates clustered together with the Australia NFNB isolates whereas the South-African SFNB isolates were grouped into a distinct cluster separate from the Australian SFNB isolates. No genetic differentiation associated with locations within Australia or South Africa could be identified.

The program Structure separated the PTT and PTM isolates into three and two groups, respectively.

DISCUSSION

Our study indicates that the genetic diversity among South African and Australian *Pyrenophora* isolates is low and that there is no clear geographical substructuring. These findings are similar to those of studies in other regions (3, 5, 6). Results produced by the two software packages NTSYS and Structure will be compared and discussed.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Hugh Wallwork and Dr Sanjiv Gupta for the isolate samples provided by them. We also would like to thank Debbie Snyman, Denise Liebenberg and Lizaan Rademeyer for their technical help in the Cengen laboratory. This project was funded by the South African Winter Cereals Trust.

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30 Incorporating host-plant resistance to Fusarium crown rot into bread wheat

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INTRODUCTION

Crown rot, caused predominantly by *Fusarium pseudograminearum* (teleomorph *Gibberella coronicola*), is a major soilborne disease problem in the wheat and barley industries. The disease is widespread and causes losses in yield and quality in Queensland, New South Wales, Victoria, and South Australia. Losses are estimated to be up to $56M in bread wheat throughout Australia. In Queensland, losses have been estimated at up to 50% in some areas and losses of 20 to 30% occur regularly, while the disease can inflict yield loss of up to 89% (1).

Breeding for resistance to crown rot has been difficult, partly due to variability associated with disease measurement, but also due to an incomplete understanding of the nature of the genetics of resistance.

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

MATERIALS AND METHODS

Of the bread wheat genotypes studied, two (Puseas and Kennedy) are susceptible and seven (2–49, CPI133814, IRN497, Lang, QT10162, Sunco, and W21MMT70) have partial resistance. The parent 2–49 is considered one of the strongest sources of resistance to crown rot currently available (3).

The seedlings were assessed for crown rot resistance in a glasshouse test, following a modification of the Wildermuth and McNamara method (4). This method is a three week duration experiment that closely mimics field infection, and is highly correlated with field results.

The approaches we took were:

- selection in targeted crosses with knowledge of the genetic model
- selection without knowledge of the genetic model
- gene pyramiding using half-sib crosses
- gene pyramiding using molecular tools (DArT).

RESULTS AND DISCUSSION

Having the genetic information available enables an informed decision to be made about the difficultly in working with particular crosses.

Many of the crosses in this study had complex epistatic models controlling crown rot resistance. A number were controlled through an additive gene model or additive x additive epistasis, which allows the resistance to be captured in a fixed line. A number of other crosses with strong resistance were controlled with dominance or dominance x dominance epistasis, meaning the resistance will not be able to be captured in a fixed line.

This information is able to guide selection of populations to advance, and explains why resistance in parent lines or segregating material alone will not guarantee resistance will be found in a fixed line. Further work is under way to compare selection in crosses with different types of epistatic control.

Selection without knowledge of the genetic control can still provide useful results, but until arriving at a fixed line there will be uncertainty about whether the disease resistance is real or the product of unfixable gene interactions.

Half-sib crosses (where the male and female have a parent in common) combining two different sources of resistance, in this case IRN497 and the synthetic wheat CPI133814, were crossed into a common agronomic background (Sunco). This can be a useful method of elevating resistance by combining diverse resistance genes. Resistance levels in the progeny are extremely high after three rounds of selection (F2 to F4), with the best showing ~30% less disease severity than 2–49.

DArT markers have been used to direct intercrosses between resistant selections from a cross of CPI133814 and IRN497, which was identified as the optimal cross for strongest crown rot resistance (from the listed parent set). DArT genotyping can identify gene differences in individuals that show the same level of resistance, enabling crosses to be made to maximise the amount of resistance genes within an individual plant. This strategy is aiming to produce a parental line for further development with elevated resistance levels beyond those currently available, rather than a variety for release, as the parents lack adaptation characteristics.

Pre-breeding selection work has commenced with the better performing crosses that include an adapted parent in the cross.

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37 A comparative study of methods for screening chickpea and wheat for resistance to root-lesion nematode *Pratylenchus thornei*

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INTRODUCTION

Several quantitative methods are available for testing resistance of crops to root-lesion nematode (*Pratylenchus thornei*) under controlled environments. This study aimed to compare growth times for wheat and chickpea cultivars and the nematode extraction procedure of Whitehead tray, (Whitehead and Hemming 1965) with shake-elution (Moore *et a*l 1992) and misting (Hooper 1986) methods. The objective was to optimise differences between susceptible and resistant chickpea lines for plant breeding purposes.

MATERIAL AND METHODS

Five chickpea and two wheat lines covering a range of resistance to *P. thornei* were selected for testing. The design consisted of 3 replicates in randomised blocks with 6 harvest times and 3 extraction methods. For each time x variety x replicate combination there were 2 pots to enable nematode comparison from one half of each pot using standard Whitehead tray method, while roots were extracted from the other half for either shake-elution or misting. Single plants were grown in pots of 330 g of steam–sterilised vertosol maintained between 22–25°C in a glasshouse on a 2 cm tension bottom–watering system. A 15 ml suspension to provide 10,000 *P. thornei*/kg soil was pipetted around the seed at planting. At each harvest of 12, 14, 16, 18 and 20 weeks, soil with roots from pots was sectioned longitudinally into halves for nematode extractions. For all 3 extraction procedures room temperatures were in the range of 22–26°C and nematodes were collected on a 20 µm sieve. Whitehead and shake-elution extractions were assessed at 1, 2, 3, 4 and 7 days while misting extractions were assessed at 4 and 7 days. Nematodes were counted in a 1-mL Hawksley slide and expressed as number of *P. thornei*/kg soil (oven-dry equivalent) or *P. thornei*/g root (fresh weight). A multi–factorial data analysis was performed using ln(x+c) where x = nematodes/kg soil and c = constant.

RESULTS AND DISCUSSION.

The Whitehead tray method extracted significantly ($P < 0.001$) more *P. thornei* than either misting or shake-elution (Fig. 1 a and b). Growing chickpeas for a longer period (18–20 weeks) than wheat (16–18 weeks) gave maximum discrimination of resistance/susceptibility in cultivars (Fig. 2). The extraction efficiency for 2 days was 70% of that at 7 days when using Whitehead trays. All the above results showed similar differences between treatments whether expressed as *P. thornei*/kg soil or as *P. thornei*/g root. The Whitehead trays were found to be less labour intensive than misting and shake-elution procedures, and more practical for assessing large numbers of plants for resistance.

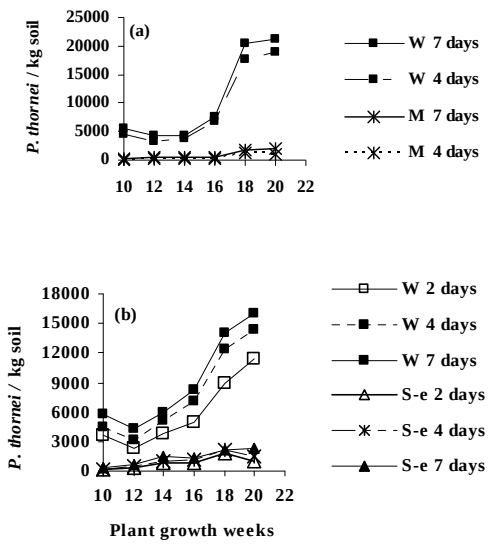

Figure 1. For each harvest the total number of *P. thornei* extracted from wheat and chickpea with Whitehead trays (W) was significantly higher ($P < 0.001$) than misting (M; Fig. 1a) and shake-elution (S-e; Fig. 1b).

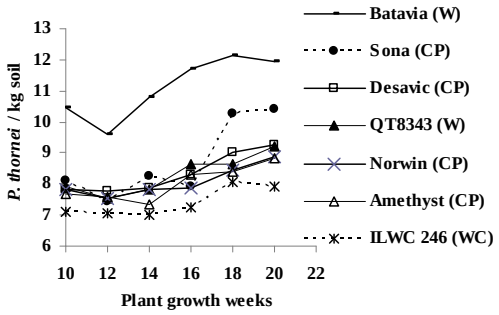

Figure 2. Longer growth periods allowed better discrimination among chickpea cultivars. Value of l.s.d. at 18 weeks and 20 weeks ($P = 0.05$) = 0.82 and 1.32 respectively. W=wheat, CP=chickpea, WC=wild chickpea.

ACKNOWLEDGEMENTS

Kerry Bell for statistical analysis and Indooroopilly Research laboratories for use of their misting facilities.

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42 A single plant test for resistance in wheat to crown rot and root-lesion nematode (*Pratylenchus thornei*)

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INTRODUCTION

Fusarium pseudograminearum, the cause of crown rot, and root-lesion nematode (*Pratylenchus thornei)* are the most serious soil-borne pathogens of wheat in the Australian northern grain region. Only one cultivar (EGA Wylie) is both tolerant to *P. thornei* and moderately resistant to crown rot. Glasshouse methods have been developed to test wheat for resistance to crown rot (Wildermuth and McNamara 1994) and root-lesion nematode (Thompson 2008) separately. This paper reports an experiment aimed to develop a single-plant method for assessing resistance to both crown rot and *P. thornei,* which would be very valuable for accelerated wheat breeding.

MATERIALS AND METHODS

Eleven reference wheat cultivars for crown rot (susceptible Puseas and Vasco, moderately susceptible Hartog, and partially resistant Gala and 2–49), and for *P. thornei* (susceptible Gatcher, Batavia and Cunningham, and partially resistant GS50a, QT9048 and Yallaroi) were tested. Five replicate 67 mm square pots received 430 g of steam-sterilised clay-loam soil moistened to 37.5% moisture (-0.1 bar), and 10 seeds of each cultivar were placed on top. Seed was covered with 100 g dry soil (5% moisture), then 0.3 g of ground barley/wheat seed colonised with *F. pseudograminearum* was added followed by 30 g dry soil. After 7 days in a glasshouse at 25°C, top watering of the pots to 37.5% moisture was commenced. After 3 weeks, soil was washed away from the seedlings and the first three leaf sheaths were rated for crown rot symptoms on a 1 to 4 scale and summed (max score = 12).

After the crown rot test, four plants from each pot, with roots trimmed to 3 cm, were planted individually in pots of 330 g steamed vertosolic soil (Irving Series), watered and inoculated with a suspension of *P. thornei* to provide 10,000/kg soil. The plants were placed in a growth room for 4 days after which permanently wilted leaf tissue was cut off. Plants were then grown in a glasshouse with temperature at 22°C and constant 2 cm soil water tension (85% moisture). The pots received three drenches with 0.1% (w/v) benlate over 6 weeks to prevent crown rot developing further. After 16 weeks, a 150 g subsample of soil and roots from the bottom half of the pots was extracted for nematodes in Whitehead trays. *P. thornei* were counted in a 1-ml Hawksley slide under a compound microscope and expressed as number/kg soil and transformed by ln(x+c) for analysis of variance.

RESULTS

The crown rot standard cultivars performed as expected, with 2–49 and Gala relatively resistant, and Hartog, Vasco and Puseas of increasing susceptibility (Fig. 1). All *P. thornei* standard cultivars were relatively susceptible to crown rot. Results for *P. thornei* are given in Fig. 2 in log units. Backtransformed values ranged from 16,150 *P. thornei*/kg soil for QT9048 to 136,380 for Puseas. The standard cultivars for *P. thornei* performed as expected with GS50a, QT9048 and Yallaroi being relatively resistant, and Batavia, Gatcher and Cunningham being relatively susceptible to *P. thornei.* Hartog produced intermediate numbers of *P. thornei* as expected from previous experiments. All of the other crown rot standard cultivars were relatively susceptible to *P. thornei.*

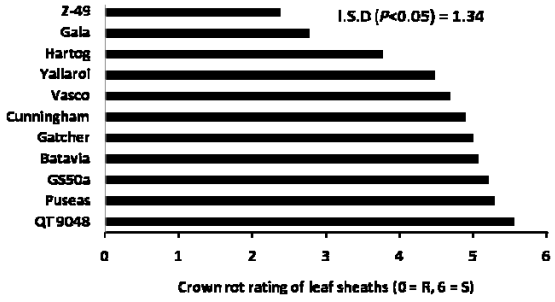

Figure 1. Crown rot ratings of seedlings

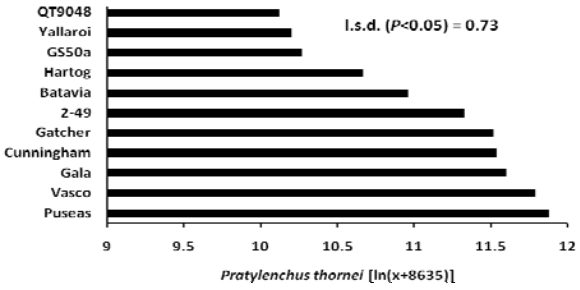

Figure 2. Number of *Pratylenchus thornei* after 16 weeks

DISCUSSION

This initial experiment shows it is possible to screen individual plants for resistance to both crown rot and *P. thornei.* The approach taken was first to test plants for crown rot by a standard method, then transplant them for a nematode resistance test. This was not ideal in that the plants suffered considerable transplanting stress and the method was labour intensive. Despite this, meaningful results were obtained for resistance to *P. thornei.* Modification of the methods should be possible to obtain an effective single plant test for resistance to both crown rot and *P. thornei* without the need to transplant.

ACKNOWLEDGEMENTS

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40 A single plant test for resistance to two species of root-lesion nematodes and yellow spot in wheat

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INTRODUCTION

Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and the stubble-borne fungal disease yellow spot (*Pyrenophora tritici-repentis*) cause substantial loss of wheat production in the Australian northern grain region. While some wheat varieties have partial resistance or tolerance to some of these diseases none has resistance to all. If varieties could be produced that combine resistance to all these diseases then the savings to the wheat industry would be very great.

Phenotyping for multiple diseases on a single plant could be a valuable method for rapidly breeding multiple disease resistant wheat varieties. A method has been developed to test for resistance to *P. thornei* and *P. neglectus* simultaneously (Huang *et al.* 2005) and in this study we extend it to include yellow spot.

MATERIALS AND METHODS

Forty-one wheat cultivars were subjected to three inoculation treatments (i) root-lesion nematodes (*P. thornei* and *P. neglectus*), (ii) yellow spot, and (iii) root-lesion nematodes and yellow spot together. Five replicates were grown as single plants in individual pots of 330 g of steam-sterilised vertosolic soil. Nematode inoculum of the two species was produced separately, and mixed in suspension prior to inoculating 5,000/kg soil of each species at sowing. The plants were grown in a glasshouse with soil maintained at 22°C and 2 cm water tension. At the 2-leaf stage, the seedlings of the yellow spot treatment were spray inoculated with field-collected *Pyr. tritici-repentis* conidia (0.45 mg conidia/mL). Inoculated seedlings were held in a mist chamber for 40 h, and then another 4 days in a growth room with sprinklers operating for 3 mins every 12 hrs, and temperature at 23.5°C. The plants were rated for combined chlorosis and necrosis of the leaves on a 1 (susceptible) to 9 (resistant) scale. All pots were returned to the glasshouse and laid out in a split block design. After 16 weeks from sowing, nematodes were extracted from the soil and roots by the Whitehead tray method. *Pratylenchus thornei* and *P. neglectus* were identified on morphology and counted under a compound microscope. Nematode numbers [after transformation by ln(x+c)] and yellow spot ratings were analysed by ANOVA. Mean values of the 41 wheat lines were used in regression analyses.

RESULTS AND DISCUSSION

There was good discrimination between wheat lines for yellow spot ratings ($P < 0.001$) and a highly significant regression relationship ($P < 0.001$) between yellow spot ratings in the presence and absence of *Pratylenchus* inoculum (Fig. 1). This indicated that a systemic resistance was not induced and that yellow spot resistant and susceptible wheats could be reliably identified in the presence of the nematodes.

The wheat cultivars inoculated with yellow spot or not were ranked similarly for *P. thornei* resistance ($R^2 = 0.7756$, $P < 0.001$) or *P. neglectus* ($R^2 = 0.484$, $P < 0.001$) or for total *Pratylenchus* ($R^2 = 0.7738$, $P < 0.001$) (Fig. 2). Numbers of *P. thornei* and *P. neglectus* in the treatment also tested for yellow spot resistance were significantly lower than in the nematode only treatment. This effect was not correlated with the yellow spot resistance rating of wheat lines. It was probably due to the changed growth conditions for conducting the yellow spot test resulting in less reproduction compared with the plants kept in the glasshouse.

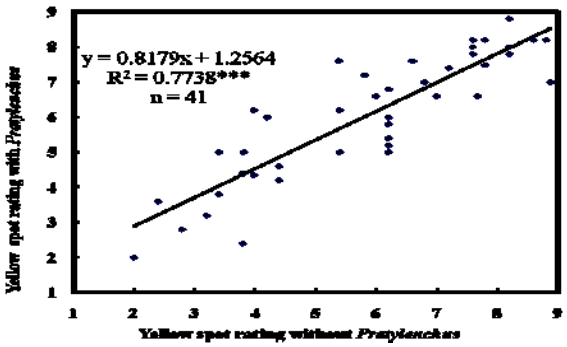

Figure 1. Highly significant regression relationship between ratings for yellow spot of 41 wheat lines when tested either without (x axis) or with *Pratylenchus* (y axis).

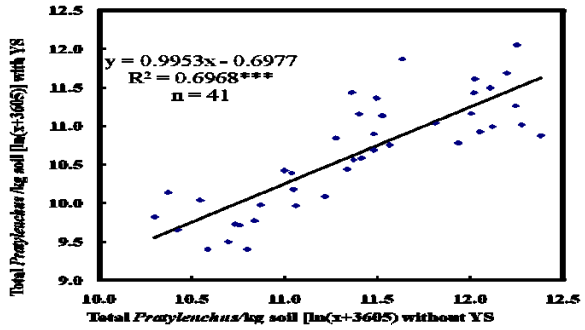

Figure 2. Highly significant regression relationship between number of *Pratylenchus* produced by 41 wheat lines when tested either without (x axis) or with yellow spot (y axis)

These results indicate that simultaneous testing of single plants for resistance to *P. thornei, P. neglectus* and yellow spot is feasible. With further refinement this method could improve the efficiency of breeding multiple-disease resistant wheats which would be of great value to Australia.

ACKNOWLEDGEMENTS

We thank Megan Brady for technical assistance.

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90 Role of nematodes and zoosporic fungi in poor growth of winter cereals in the northern grain region

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INTRODUCTION

The endoparasitic root-lesion nematodes *Pratylenchus thornei* and *P. neglectus* and the ectoparasitic stunt nematode *Merlinius brevidens* occur widely in the northern grain region of Australia. Grain loss in wheat has been well characterised for *P. thornei* in the northern region (Thompson *et al* 2008) and for *P. neglectus* in the southern and western regions (Vanstone *et al*, 2008). Information on the role of *Merlinius brevidens* is sparse although it has been shown to cause yield loss of wheat in the USA (Smiley *et al.* (2006), particularly when associated with the zoosporic fungus *Olpidium brassicae* (Langdon *et al.*1961). Following diagnosis of high populations of *M. brevidens* associated with poor crops of winter cereals in 2007 a glasshouse experiment was conducted in 2008 to explore further the reasons for the poor growth

MATERIALS AND METHODS

About 50 kg of soil was collected (on a grid of 36 positions within a 1,920 m^2 area) from each of nine fields in the northern grain region located from Garah in northern NSW to Wondai in Qld. These sites were selected on the basis that *M. brevidens* and/or *Olpidium* sp. had been detected in poorly growing cereals in the field or on the farm previously. The soil from each site was mixed, sieved and about half was partially sterilised by steam at 70ºC for 45 min. Quantities of each soil (330 g OD equivalent) were mixed with 1 g of Osmocote® [Native Gardens plus micronutrients (17–1.6–8.7 NPK)] slow-release fertiliser and placed in 5 cm-square plastic pots suitable for bottom watering. Eighteen pots of each of sterilised and unsterilised soil were prepared to allow for growing 3 replicates of 3 cereals (wheat cv. Strzelecki, barley cv. Grout and oats cv. Coolibah) at 2 moisture tensions (2 cm and 7 cm) and 2 harvest times (8 and 16 wks). The pots were placed on strips of capillary matting for each soil type and on separate benches for sterilised and unsterilised soil and for the two water tensions. A single plant per pot was grown, with the glasshouse temperature kept below 25ºC by evaporative coolers. At each harvest, plant tops were dried at 85ºC for 4 days and weighed. Soil and roots were removed from the pots, photographed and split longitudinally. Roots were extracted from one half of the pots, blotted, weighed, and a subsample stained with trypan blue. Soil and roots from the other half were broken into pieces <1 cm and a subsample extracted for nematodes by the Whitehead tray method.

RESULTS AND DISCUSSION

All three cereals responded to soil sterilisation with mean plant dry weight in unsterilised soil ranging from 44 to 95% of that in sterilised soil when grown at 2 cm water tension (Fig. 1). The effects were relatively similar at the two water tensions of 2 and 7 cm (Fig. 1). Root systems were considerably reduced in the unsterilised soil compared with the sterilised soil.

Observations of the stained roots under the microscope showed the presence of zoosporangia and encysted zoosporangia similar to those of *Olpidium radicale* and *Olpidium brassicae* as well as *Pythium* oospores. High populations of *M. brevidens* and of *P. thornei* were present in samples extracted at 16 weeks.

The work is still in progress and this is a preliminary report.

It does, however, indicate poor root health of cereals growing in the northern region that may be due to the combined effects of nematodes and zoosporic fungi.

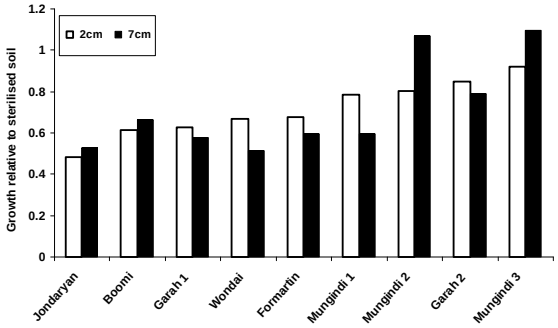

Figure 1. Response at 8 weeks in growth at two water tensions of winter cereals (mean of wheat, barley and oats) to sterilisation of soil from 9 farms in the northern grain region

ACKNOWLEDGEMENTS

We thank graingrowers for permission to sample their fields and GRDC for funding.

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41 Sources of resistance to root-lesion nematode (*Pratylenchus thornei)* in wheat from West Asia and North Africa

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INTRODUCTION

The root-lesion nematode *Pratylenchus thornei* occurs widely in the northern grain region (northern NSW and southern and central Qld) causing considerable economic loss in wheat production. Current management tools are hygiene with farm machinery to prevent transfer of infested soil, crop rotation and growing tolerant wheat varieties (Thompson *et al.* 2008). More effective control of the nematode populations could be achieved if resistant cultivars were available.

To obtain novel sources of resistance we tested two collections of wheat from the West Asia and North Africa (WANA) region. The A.E. Watkins Collection was made in Cambridge, UK, in the late 1920s and early 1930s with landrace wheats from many countries of the world (Miller *et al.* 2001). The R.A. McIntosh Collection was made in 1993 at University of Sydney with wheats from WANA countries for studies on rusts and flag smut

MATERIALS AND METHODS

Wheat Accessions. The WANA wheats tested comprised 148 bread wheat (*Triticum aestivum*) and 139 durum wheat (*Triticum turgidum* spp. *durum*) accessions from the Watkins Collection and 59 bread and 43 durum accessions from the McIntosh Collection.

Resistance Experiments. Initially each of the above collections was tested for resistance to *P. thornei* in two separate glasshouse experiments that included the reference standards GS50a (a partially resistant bread wheat) and three susceptible wheat varieties Gatcher, Suneca and Potam. A number of bread and durum wheat accessions that produced nematode numbers not significantly different from GS50a were retested for resistance in a third experiment.

Resistance test methods. The wheat accessions were grown as 3 replicates in pots of steamed vertosolic soil (1 kg soil in Experiments 1 and 2 and 650 g in Experiment 3), inoculated with *P. thornei* at a rate of 2,500/kg soil. The soil was fertilised to provide N, P, K, S, Ca and Zn and was watered to pF2 (56% moisture). The experiments were laid out in randomised blocks in an evaporatively cooled glasshouse. In Experiment 3, the soil and root temperature was kept at 22°C with pots in glasshouse waterbaths. After 16 weeks growth, one half of the soil and roots was removed, broken to < 1 cm manually and 150 g extracted for nematodes in Whitehead trays. Nematodes were counted under a microscope and expressed as *P. thornei/*kg soil (oven dry equivalent). Data were transformed by ln(x+1) for ANOVA and calculation of *F*l.s.d. Backtransformed means and reproduction factors (RF = final number of *P. thornei/* initial number) were calculated.

RESULTS AND DISCUSSION

Experiments 1 and 2. As a group, the bread wheats were significantly ($P < 0.001$) more susceptible to *P. thornei* than the durum wheats with backtransformed means for *P. thornei*/kg soil of 52,051 for bread wheats and 38,560 for durum wheats in the Watkins Collection, and 34,200 for bread wheats and 21,268 for durum wheats in the McIntosh Collection.

Experiment 3. Thirteen WANA bread wheats (Fig. 1) and 10 durum wheats (data not shown) had *P. thornei* numbers that did not differ from GS50a in two experiments. All Australian bread wheats were susceptible whereas the two Australian durums were as resistant as GS50a.

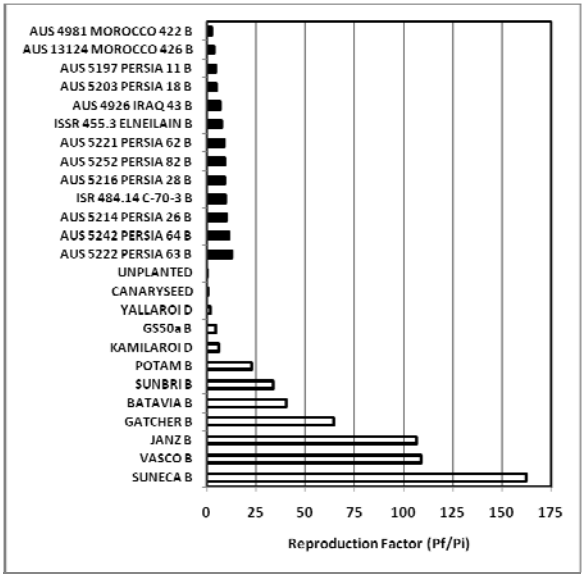

Figure 1. Reproduction factor of WANA bread wheats (black bars) that did not differ significantly from GS50a in comparison with reference standard wheats (open bars) from the northern grain region. The letters B and D after names indicate bread and durum wheats respectively.

The identification of additional sources of resistance in bread wheat to *P. thornei* provides greater options for producing Australian wheat varieties with greater levels of resistance to *P. thornei* than in current varieties.

ACKNOWLEDGEMENTS

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