THE ROVIRA RHIZOSPHERE SYMPOSIUM

CELEBRATING 50 YEARS OF RHIZOSPHERE RESEARCH

EDITORS
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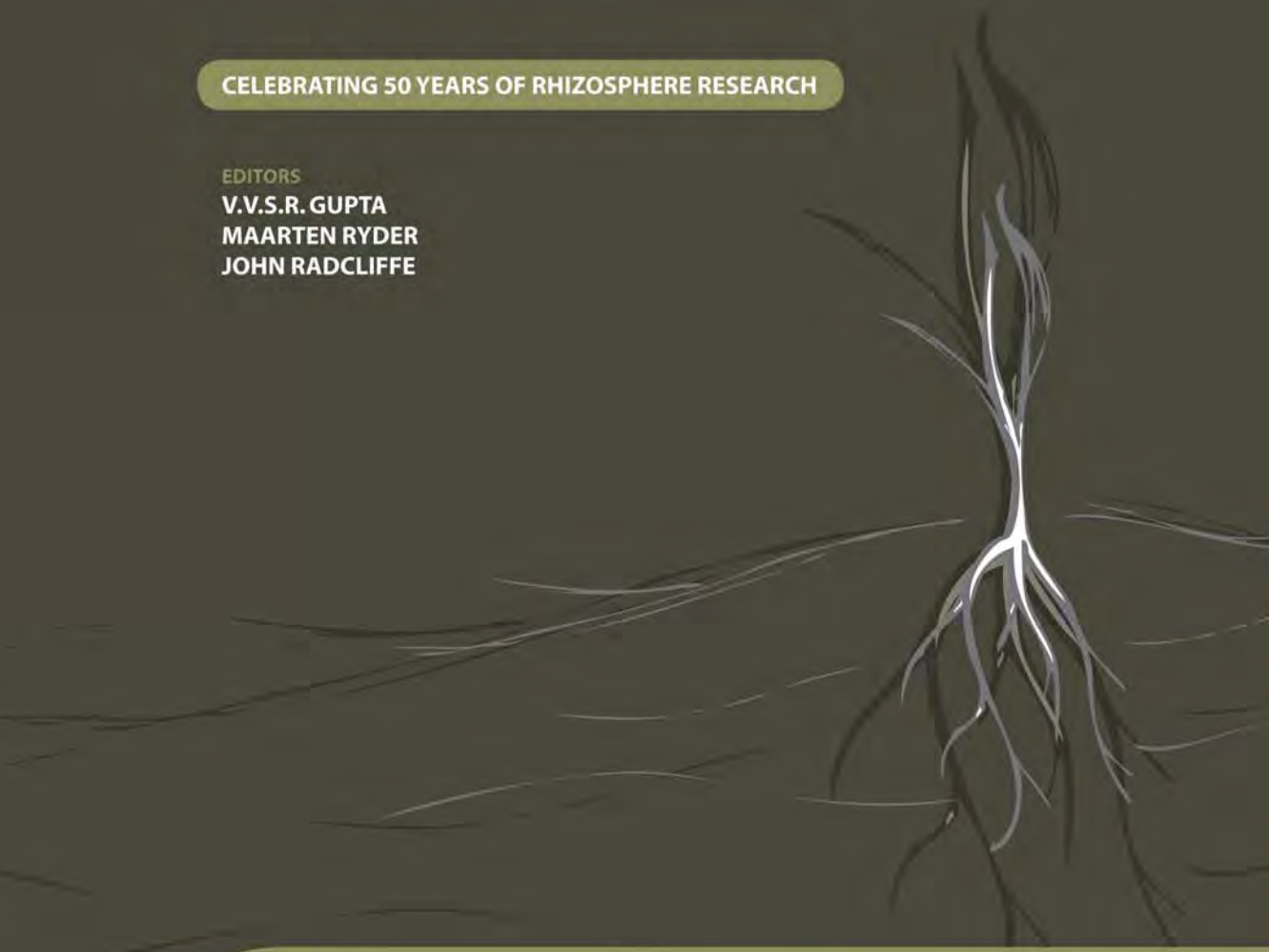
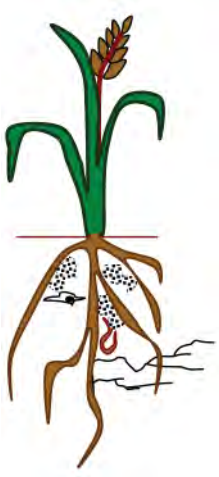


Proceedings of a symposium 15 August 2008
SARDI Plant Research Centre, Waite Campus, Adelaide
The Crawford Fund



THE ROVIRA RHIZOSPHERE SYMPOSIUM

Celebrating 50 years of rhizosphere research

A festschrift in honour of Albert Rovira AO FTSE

Editors

V.V.S.R. Gupta
Maarten Ryder
John Radcliffe

Friday 15 August 2008
SARDI Plant Research Centre
Waite Campus, Adelaide

2010

THE CRAWFORD FUND
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The Crawford Fund

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Telephone: (612) 6285 8308
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 viii + 136 pp.
ISBN 978 1 921388 07 1
Production editor: Alan Brown
Cover: Whitefox Communications
Printing: Goanna Print, Canberra

Dedication

To Dr Albert Rovira AO FTSE in honour of his 80th birthday
and
fifty years of rhizosphere research and leadership

Prior to his formal retirement, Dr Rovira was a Chief Research Scientist in the CSIRO Division of Soils and foundation Director of the CRC for Soil and Land Management, which was one of the pioneering 'first round' Cooperative Research Centres.

He continues to mentor current students and scientists and is still publishing.

Dr Rovira has been the Coordinator of the ATSE Crawford Fund in South Australia since its inception.

Organising Committee

Vadakattu V.S.R. Gupta
Maarten Ryder
Rosemary Warren
John Radcliffe
Paul Harvey
Kathy Ophel-Keller

Acknowledgements

The organisers wish to thank the following organisations and people
for their generous support and assistance:

CSIRO
SARDI
The Crawford Fund
Stasia Kroker, Jan Mahoney and Suellen Slater of CSIRO
Geetha Vadakattu
Cathy Reade and Alan Brown of The Crawford Fund
Martha Hawes of The University of Arizona
Reviewers of the manuscripts

Frontispiece 1. International participants with Albert Rovira. Left to right: Richard Smiley, Albert Rovira, Sina Adl and Jim Tiedje

Frontispiece 2. 1–Emma Leonard, 2–Jane Greenslade, 3–Sina Adl, 4–Fitri Widiantini, 5–Evelina Facelli, 6–Tanya Bernardo, 7–Eileen Scott, 8–Laura Thompson, 9–Albert Rovira, 10–Herdina, 11–Vadakattu Gupta, 12–Denis Blight, 13–Elizabeth Drew, 14–John Radcliffe, 15–Susan McKay, 16–Bob Hannam, 17–Duan Tingyu, 18–Monique Shearer, 19–Lyn Abbott, 20–Richard Lardner, 21–Megharaj Mallavarapu, 22–Chris Franco, 23–Graham Stirling, 24–Sandy Dickson, 25–Richard Smiley, 26–Doug Reuter, 27–Marcus Hicks, 28–Stasia Kroker, 29–Rowena Davey, 30–Diana Walter, 31–Rabina Sasa, 32–Neil Andrew, 33–Helle Christophersen, 34–Graeme Jennings, 35–Matthew Ayres, 36–Ray Correll, 37–Nigel Wilhelm, 38–Maarten Ryder, 39–Rohan Rainbow, 40–Rob Velthius, 41–Richard Burns, 42–Jim Tiedje, 43–Alan McKay, 44–Paul Harvey, 45–Alan Buckley, 46–Sally Smith, 47–Steve Barnett, 48–Roger Swift, 49–Richard Winder, 50–Simon Anstis, 51–Ron Kimber, 52–Neil Venn, 53–Neil Smith, 54–Murali Naidu, 55–Andrew Smith, 56–Richard Simpson, 57–Glen Bowen, 58–Geoff Baker, 59–Mark Seeliger, 60–Rosemary Warren, 61–Bernard Doube, 62–Kathy Ophel-Keller

Preface

A symposium to honour the 80th birthday and scientific contributions of
Dr Albert Rovira AO FTSE
and to explore the latest developments in rhizosphere research

A symposium was held in Adelaide on 15 August 2008 to celebrate the 80th birthday of Dr Albert Rovira and more than 50 years of his research and leadership in the science of agriculture.

The *rhizosphere* is that dynamic hub of biological activity that surrounds plant roots. Since it was defined in 1904 by Dr Lorenz Hiltner, the rhizosphere has been one of the most fascinating habitats for research in basic and applied microbiology. Our understanding of rhizosphere organisms and the many roles they play in plant growth, crop production and ecosystem health has developed tremendously. Yet there are still considerable challenges ahead in our attempts to harness the rhizosphere for improved crop growth and sustainable production. Fortunately, advances in other fields of science and technology (molecular biology, microscopy and mass spectroscopy) allow us to make continuing progress in understanding the complexity and function of the rhizosphere. This might lead to the development of 'designer rhizospheres' for specific purposes.

Pioneering work that combined the latest concepts with new analytical techniques in microbiology, plant physiology and microbiology was done by Dr Albert Rovira in the 1950s. His seminal papers on the rhizosphere, published in 1956, started a new chapter in rhizosphere research. Since that time, Australian scientists have been very well known internationally as part of research effort to understand and manage the rhizosphere for improved crop production. The importance of Albert's contribution is summed up in a recent statement by Professor Martha Hawes (University of Arizona), who said that 'the longer I'm in rhizosphere research the more I appreciate his vision and work, and it still remains rather singular. ...'

In this volume we have assembled keynote papers presented by leading national and international scientists at the symposium. The paper by Dr Richard Burns highlights some of the significant contributions of Albert's research and coins the term *Rovirasphere*. The papers in these proceedings consider a variety of aspects of rhizosphere research on microbial and faunal communities and their interactions with plant growth and production, and ranging from beneficial to deleterious. Some of the latest developments in DNA and RNA technologies allow us to unravel interactions at the microsite level and, by identifying some of the critical steps and processes involved in regulation, we are optimistic that we will continue to scale-up our understanding of what occurs at the root–soil interface to practical applications in the field and further to the agro-ecological zone. Albert's later work on soil biology led to many practical improvements in cropping systems in Mediterranean and temperate regions. A contribution from the farming community is included in the proceedings in recognition of this important part of Albert's career.

Prior to his formal retirement, Albert Rovira was a Chief Research Scientist in the CSIRO Division of Soils and was the foundation Director of the Cooperative Research Centre for Soil and Land Management. Since retirement he has continued to mentor students and scientists in Australia and also in other countries such as China, and is still writing and publishing. His works have been cited an average of 56 times per year for over 56 years.

Albert has made another significant contribution in helping to establish The Crawford Fund in South Australia. He has been the South Australian Coordinator since its inception here in 2000 and has maintained a vital interest in the projects and people who have been supported by the Fund.

We are pleased to present this series of papers as the proceedings of the 'Rovira Rhizosphere Symposium' and thank all who have made contributions in organising the symposium, and in writing, editing and producing this volume.

Vadakattu V.S.R. Gupta
John C. Radcliffe
Maarten H. Ryder

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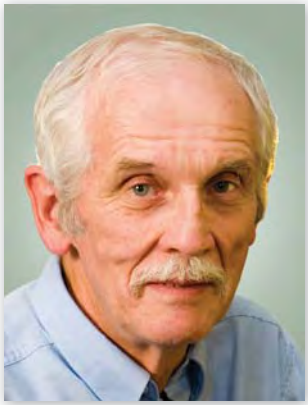

Albert Rovira and a half-century of rhizosphere research

Richard G. Burns[1]

Abstract

The recognition of the soil-root interface as a zone of high microbial activity is over a century old, but a rational and evidence-based confidence in the possibility of its manipulation is much more recent. Today the major emphases in fundamental rhizosphere research are towards describing microbial diversity, translating the chemical language that plants and microbes use and deciphering how the soil properties influence communication. Successful modification of the rhizosphere for plant growth promotion and the retention or recovery of soil quality will be dependent on a clear appreciation of the many and often subtle relationships that plants have with each other as well as with the vast numbers of harmful and beneficial soil microbes in their vicinity. The rapidly growing catalogue of plant and microbial genomes and a fast-expanding knowledge of how signals are generated and perceived are playing a big part in our improving understanding. Also important is an acceptance that plant-microbe relationships have co-evolved and are continuing to do so as land use, agricultural practices, soil quality, climate and crop selection change. Albert Rovira's writings over the past 50 years have played a major role in our exploration and explanation of the rhizosphere. His contributions are as varied as they are profound and encompass technical advances and experimental investigations of the rhizosphere (root exudates, microbial enumeration, ultrastructure, nutrient accession, virulence factors, etc.) as well as applied aspects closely related to plant health and soil fertility (biological control of fungal pathogens and nematodes, ammonification and nitrification, pesticide affects, soil structure, climatic impacts, soil management, etc.). Rovira's research is that rare combination of fundamental studies and their successful application to the real (and often harsh) world of agriculture. Some of the many ways in which Albert Rovira's discoveries and ideas have provided signposts for current investigations and, ultimately, for accomplishing the sustainable management of soil are summarised in this article.

Introduction

Over the past century rhizosphere research and its real or anticipated application to agriculture has enjoyed periods of popularity and long spells of stagnation. Today is a time of great excitement and optimism. A search of Web of Science reveals that more than 1000 papers concerned with the rhizosphere were published in 2008 and in excess of 1500 in the first nine months of 2009. Eleven years ago, during the whole of 1998, there were 366 and twenty years ago there were just 50. (When Albert Rovira began his career there were less than ten rhizosphere papers a year!) In fact, throughout much of the previous century soil biologists shied away from including plants in their experiments: soil is complicated enough without adding

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the ever-changing influence of a growing plant. But nowadays a more holistic real-world attitude prevails and plants are acknowledged as essential components of most soil biology research programs; this is reflected in the surge of exciting papers devoted to all things rhizosphere.

Why this dramatic change? One explanation is historical: the centenary of Hiltner's 1904 concept of the root-soil interface (albeit restricted to bacteria and legumes) prompted many celebratory meetings, most notably in Munich in 2004 (Wenzel 2005; Jones *et al*. 2006). At that time the need for regular international rhizosphere meetings was recognised and experts reconvened in Montpellier in 2007 (Jones *et al.* 2009a) and will do so again in Perth in 2011. There have been many other recent gatherings to discuss aspects of plant-microbe-root communication: Lapland, Finland (2005), New Hampshire, USA (2008) and Brisbane, Australia (2008) (Schenk *et al*. 2008). A second impetus was provided by growing concerns about the negative impacts of climate change and any number of anthropogenic and natural stresses on crop productivity. Suddenly there was a need to increase our knowledge of how soils and plants interact in order to ameliorate some of these stresses, most pressingly in relation to temperature and rainfall patterns. Thirdly, and with perfect timing, a new tool box jammed full of useful techniques has appeared. These include sensitive analytical equipment, advances in microscopy and molecular biology, microsite sampling, some clever modeling and new ideas about scale-up, plus the evaluation of complex sets of data using, for example, principal component analysis.

Many of these techniques were developed in other fields but they are now being enthusiastically adopted and modified by soil microbiologists, and are driving research at an accelerating pace. In particular, there is no doubt that the DNA sequencing of plants and microbes and the increasing number of ways to study the proteomics, transcriptomics, metabolomics and metagenomics of soil populations are powerful molecular weapons in our armory as we struggle to understand that most labyrinthine of all environments, the soil. The techniques include a plethora of molecular methods for extracting DNA, rRNA and mRNA from soil and then amplifying, fingerprinting, cloning and sequencing (Oros-Sichler *et al*. 2007; Sharma *et al.* 2007). As a consequence, it is becoming increasingly possible to accurately describe and compare both the genotype and the phenotype of the hugely diverse microbiota of soil and to monitor how it responds to the growing plant. It is no longer necessary to be daunted by the fact that as many as 95% of soil microorganisms have never been grown in pure culture, even though this may be a consequence of our poor knowledge of microbial nutrition rather than there being vast numbers of species that are inherently non-culturable. But nowadays we do not need to grow bacteria and fungi on agar in a Petri dish to be able to ascertain their immense process capabilities and to gauge their importance in soil metabolism. In addition, the movement, colonisation and activities of both indigenous and introduced microorganisms can be studied using the powerful new developments in, for example, autofluorescence and confocal laser scanning microscopy (Bloemberg 2007).

This combination of technical carrots and environmental sticks has given us real hope that we are on the verge of manipulating the soil environment in a rational and predictive manner. The novel methods have also given us the confidence to think imaginatively about the types of interactions that are occurring in the root region and to embrace the idea that there is a complicated language used by plants and microbes. The chemical cross-talk that takes place between plants and plants, microbes and microbes, and plants and microbes, and how that conversation is translated into action is further complicated by the chemical and physical characteristics of the soil.

Recently published books (Cooper and Rao 2006; Cardon and Whitbeck 2007; Pinton *et al*. 2007) and the very large number of insightful review articles (e.g. Bais *et al*. 2006; Gregory 2006; Kraemer *et al.* 2006; Bouwmeester *et al*. 2007; Steinkellner *et al*. 2007; Complant *et al*. 2008; Jackson *et al*. 2008; Jones *et al.* 2009b; Raaijmakers *et al.* 2009) are a testimony to the recent surge in interest in rhizobiology.

Albert Rovira has been in the vanguard of rhizosphere research for more that 50 years and his pre-eminence in this far reaching topic is unquestioned. His influence is profound and prompts the coining of a new soil biology noun: the *rovirasphere*. This is defined as 'the area of soil-plant-microbe research and thinking influenced by the intellectual outputs and published products of Albert Rovira'. In the bibliometric world we live in this influence is well illustrated by the large number of Rovira's

publications that have been cited over 100 times, the *Nature* papers, various indices (Hirsch, Egghes's g, Impact Factor, etc.), and two papers designated as Citation Classics by the Institute for Scientific Information (Bunt and Rovira 1955; Rovira 1969). But this is only a snapshot and the rovirasphere is also exemplified by the large number of distinguished soil biology researchers that Rovira worked with and influenced. In a long list these include Richard Campbell, Jim Cook, Jennifer Parke, David Weller, Martin Alexander, David Barber, Ed Newman, Richard Smiley, Keith Martin, Maarten Ryder, Jean-Alex Molina, David Roget, Clive Pankhurst, Jack Warcup, Ralph Foster and Rovira's perennial brother-in-arms, Glynn Bowen. Together these two have published a dozen or so major papers and a number of highly influential reviews; they could be seen as the Lennon and McCartney of rhizosphere research!

Rovira and Bowen, along with Jeff Ladd, Ken Lee, Alan Posner, Malcolm Oades, Dennis Greenland, Keith Martin and together with Ray Swaby's inspired recruitment, were responsible for putting soil biology research at the CSIRO, Adelaide, on the world map in the 1960s: a position it held for almost 40 years. These are the scientists and the international face of the Urrbrae group, but the influence spread way beyond this to the practitioners—the farmers who saw Albert Rovira, in particular, as someone who understood their problems and was carrying out research that was directly aimed at making their struggle with the harsh Australian climate and the soil pests that decimate the crops a little easier. Albert strode from laboratory to glasshouse to field with a confidence and ease borne of a love of fundamental research combined with an empathy with the farmer. One brief quote that sums this up in an understated way says 'Albert Rovira is a highly respected farmer-friendly soil scientist'. The hugely influential 30 years of Avon trials are a wonderful example of this and are described elsewhere in this volume.

Rovira's research

The scope of Rovira's research is reviewed and represented by many of the papers in this special publication and will only be cherry-picked here. It is dominated by three major microbial enemies of Australian agriculture: the fungi *Gaeumannomyces graminis* (take-all of wheat) and *Rhizoctonia solani* (cereal root rot) and the root- invading eelworm *Heterodera avenae* (cereal cyst nematode). These pests, individually or in combination, will decimate crops and reduce yield if left unchecked by pesticides and other management strategies. The precise impact of these diseases was shown by comparing the yields in soils fumigated to remove the pathogens and this provided a reliable base from which to assess various control measures (Rovira and Bowen 1966). These amelioration attempts have run the gamut from the isolation, purification and identification of putative biocontrol agents to their application and assessment (Cook and Rovira 1976; Kerry *et al*. 1984; Rovira and McDonald 1986; Brisbane and Rovira 1988; Ryder *et al*. 1999; Altman *et al*. 1990). The rationale behind much of this phytopathological research and its relevance to modern sustainable agriculture are well described by Rovira himself in his 1989 Daniel McAlpine Lecture (Rovira 1989). It is noticeable that adopting modern agricultural practices, such as the use of knock-down herbicides, no-till and direct drilling of seeds, needs careful thought and thorough investigation if they are to be compatible with controlling these potentially devastating root diseases. Soil-available phosphate (Simon and Rovira 1985) and manganese (Wilhelm *et al*. 1988) also affect the expression of these plant pathogens.

The body of what might be described as Rovira's fundamental research is impressive and includes such topics as quantifying rhizosphere microbes (Brisbane and Rovira 1961) and their enzyme activities (Ridge and Rovira 1971; Neate et al. 1987), measuring and identifying root exudates (Rovira and Ridge 1973) and their sites of release along the growing root (McDougal and Rovira 1970), defining plant–plant interactions (Newman and Rovira 1975), recording nutrient accession and root uptake (Rovira and Bowen 1968), assessing essential nitrogen transformations including ammonification and nitrification (e.g. Molina and Rovira 1964; Doxtader and Rovira 1968) and visualising root and rhizosphere ultrastructure. With regard to the last-mentioned, the work by Foster and Rovira (1976) that used the electron microscope to observe and record the microbial colonisation of wheat roots and how this changes with the development of the plant was a landmark in rhizosphere science. And this is far from a definitive list; you would need to add herbicide decomposition, fungal virulence factors, earthworms, rhizobia and many others to

your search to get the full picture of Albert Rovira's research vocabulary.

Even a cursory reading of Rovira's papers reminds you time and time again of an obvious yet important message: soil is a multi-faceted environment. Thus, whilst a conventionally designed and conducted laboratory experiment in which most of the variables are controlled may provide much useful information, it is not the 'real world'. Recognising this, and rather than studying the 'effect of A on B', Rovira and his co-workers opted frequently for the 'effects of A, B, C and D, individually or in combination, under conditions E and F, in the laboratory, greenhouse and field'! There are many examples of this daunting yet pragmatic approach to soil research, for example: *Effect of sowing point design and tillage practice on the incidence of rhizoctonia root rot, take-all and cereal cyst nematode in wheat and barley* (Roget *et al*. 1996). This paper looks at conventional cultivation and three types of seed drilling, three diseases and two crops — in four field experiments over three years (I will leave it to the reader to calculate the number of treatments and controls). Yet this is exactly the sort of information that the farmer needs to allow him to make judgments appropriate to his particular circumstances.

Periodically Rovira, alone or together with Bowen, summarised and synthesised the state of the art in rhizosphere research and its applications. Four such reviews stand out (Rovira 1965, 1969; Bowen and Rovira 1976, 1999) and these have become benchmarks in the subject. Scientists studying the environment of the plant root would be well advised to use these as a starting point for their research because they are packed with ideas and intelligent speculation about what goes on underground. There are many important messages that emerge from reading these seminal reviews and just two of them are recorded here. Both may appear obvious in 2009 but they were not when the reviews were published and they still have a strong resonance today. The first is that root exudates are an integral component of soil chemistry, physics and biology and have major and multifarious effects on nutrient availability and plant health and growth. The second is that the quality and quantity of these exudates change with plant species, plant age and stage of development, soil type and its management, the resident micro- and macro-flora, and climate. In other words the rhizosphere is dynamic in terms of its processes and properties both in space and time. This may read as a rather daunting set of characteristics and, until perhaps five years ago, the possibilities for resolving the rhizosphere mysteries were in the realms of the imagination — and conference workshops. Not so now.

What makes rhizosphere research so interesting?

It has been variously estimated that between 5% and 40% of photosynthetically fixed carbon is released by the plant root (Cheng and Gershenson 2007). Although most of these measurements have been made using annuals and sometimes the methodology is questionable, this is a level of apparent inefficiency to which you would be hard pressed to find an explanation. Of course, what the plant is doing, either directly or in response to its environment, is to release carbon, nitrogen and energy sources that will stimulate the resident microbial population as the roots travel horizontally and vertically through the soil. The chemical nature of these rhizodeposits range from the comparatively simple low-molecular-mass structures (amino acids, fatty acids, phenolics, hexose and pentose sugars) to complex macromolecules (polysaccharides, proteins, nucleotides, antibiotics) and root cells and debris (Table 1). These plant products may act as general or selective nutrient sources inducing the proliferation of those microbial strains already present in horizons through which the developing root proceeds. On the other hand, soluble exudates may diffuse away from the plant and set up gradients which stimulate the movement of microbes from the wider soil towards the plant root where they can benefit from a comparatively copiotrophic location. But carbon, nitrogen and energy for metabolism and growth are not the only contributions that plant exudates make to microbial life in soil.

Once microbes are close to the root surface, a large number of other chemicals that function as signal molecules (Table 1) come into play and orchestrate further changes in both the plants and the microorganisms. These processes may result in the formation of complex and structured microbial communities at the root surface and their retention within biofilm structures (Danhorn and Fuqua 2007; Rudrappa *et al*. 2008). Yet other chemicals may trigger intimate extra-, inter- and intra-cellular relationships.

Table 1. Some plant rhizodeposits and microbial secretions, and their functions in soil

Material
• Monosaccharides, polysaccharides, amino acids, aliphatic acids, aromatic acids, fatty acids
• Proteins, peptides, enzymes, vitamins, nucleotides, purines, phenolics, antibiotics
• Alcohols, alkanes, sterols, terpenes
• Siderophores: hydroxamic acids, mugineic acids
• Root debris, border cells, root cap cells
• Inorganic ions: HCO_3^-, OH^-, H^+
• Gases: CO_2, H_2, ethylene
• Signal molecules: flavonoids, p-hydroxy acids, quinones, cytokinins, phytoalexins, coumarin, rosmarinic acid, salicylic acid, dodecoic acids, furanosyl diesters, phenypropanoids, napthoquinones, jasmonic acid, lactones, strigalactones, lectins
Functions
• Carbon, nitrogen and energy sources
• Acidity and alkalinity regulators
• Metal ion ligands
• Soil genesis and structure
• Water capture and retention
• Attraction of beneficial microbes
• Repulsion or inhibition of harmful microbes
• Induction of plant growth promoting activities
• Adhesion and biofilm formation
• Penetration and cellular integration of symbiotic bacteria and fungi
• Inhibition of competitive and invasive plants

Plant growth promotion takes many forms but is almost inevitably the direct or indirect consequence of plants and microbes detecting and then responding to each others presence. It is becoming increasingly apparent that plant roots and soil microbes have multiple ways of perceiving their immediate environment and reacting accordingly. What usually follows from the initial recognition is a cascade of events, although it is often difficult to see whether the plant or the microbes are the dominant partners in the relationship. At its most developed, the signals and responses give rise to intimate, intracellular and long-term associations (e.g. rhizobia and legumes, mycorrhizae) and these have been recognised and exploited in agriculture, horticulture and forestry for many years (Drinkwater and Snapp 2007). Other apparently more casual and sometimes non-specific relationships which benefit the plant may offer protection against phytopathogens, help in the scavenging of nutrients, stimulate soil genesis, improve soil structure, generate phytohormones, improve hydraulic continuity and water retention, and even inhibit the establishment and development of adjacent and potentially competitive plants.

We have known about plant enzymes such as phosphatases, amylases, invertases and proteases for many years, and these may stimulate many processes in soil that lead directly to the release of nutrients from either organic macromolecules or those that are attached to clays and humates and, at least temporarily, in a non-bioavailable state. Of course, these plant enzymes are only a fraction of the total extracellular catalytic complement of soil. Microbes secrete enzymes in abundance and these are an essential component of the soil's capacity to transform and mineralise the vast number of organic substrates of plant, animal and microbial origin (Burns and Dick 2002; Burns 2008).

In addition to the chemically recognisable exudates, the number of plant metabolites that are secreted into the rhizosphere is in the hundreds, and it is believed that many of these have signaling functions. In fact, secondary metabolites may number in the thousands and it is probable that some are accumulated inside the plant and only released in response to appropriate messages from the soil. The dialogue that takes place in the rhizosphere involves plants talking to plants, microbes and microfauna, microbes talking to microbes, and microbes talking to plants. Chemical messengers may be targeting individual genotypes and processes or have a more general impact on microbial community dynamics. It is this continuous chemical chatter that we are at last beginning to decipher, although the subtlety of some of these interactions is astounding. For example, different enantiomers of catechin generate different responses in adjacent plants and microorganisms (Bais *et al*. 2005) and the process of racemisation (and therefore the nature of the message) may take place within the roots or post-exudation or even be mediated by certain rhizosphere microorganisms.

Many successful bacterial pathogens, especially those of the genera *Erwinia*, *Agrobacterium*,

Xanthomonas and *Pseudomonas*, mount successful attacks on plant roots only after their cell densities have reached an appropriate level. This is known as quorum sensing (He and Zhang 2008) and serves to inform the microbial cells that there are enough of them to warrant a serious attack on the plant. What then follows is that virulence factors (e.g., root wall degrading enzymes) are transcribed and secreted and used collectively and at elevated concentrations against their target root cells. This appears to be very effective, but plants have many ways of fighting back (Jones and Dangle 2006). For instance, plants may produce enzymes that disrupt microbial communication (quorum quenching) by degrading quorum sensing peptides and acyl homoserine lactones. Plants may also secrete chemical mimics that fool the pathogenic bacteria into generating invasive enzymes long before the population is dense enough to be effective (Faure and Dessaux 2007). This ongoing evolution of attack and defense mechanisms is taken to the next stage by some successful pathogens that actually block the synthesis and secretion of plant defence chemicals. Unsurprisingly, many microbial processes that are beneficial to plants have quorum sensing at the centre of their interactions (Cooper 2007).

The next phase of rhizosphere research

Plants and soil microbes have co-evolved. In fact, fossil evidence suggests that the success of the very first land plants was dependent on mycorrhizal associations (Taylor and Krings 2005) that served to extend the root capture zone and scavenge distant nutrients. Interestingly, the process of legume nodulation may have come along much later. The beneficial associations, such as those resulting in enhanced phosphorus capture and nitrogen fixation, are reasonably well understood although the process of nodulation is influenced by rhizobacteria other than rhizobia and the tripartite association between rhizobia, mycorrhizae and plants is important to most legumes (Johansson *et al*. 2004). There are many opportunities to improve the availability and plant capture of phosphate and nitrogen in impoverished soils, and we should expect continuing advances in this area.

There is a complacency about the lasting effect of shocks on the microbial world because of the well known robustness and resilience of bacterial and fungal communities, but wake-up calls are becoming loud and piercing. Rising levels of atmospheric carbon dioxide are predicted to have a profound effect on our climate and this will be reflected in changes to many soil properties. At the very least, any increase in photosynthesis will give rise to changes in root exudation patterns and to shifts in rhizosphere microbial community diversity and function (Drigo *et al*. 2008). The International Panel on Climate Change has predicted recently that climate change will increase the susceptibility of plants to pests and diseases. This has been commented on by many (e.g., Zavala *et al*. 2008) and understanding how plants defend themselves against attack and how these processes can be manipulated is of increasing importance in 21st century agriculture. We already know that long-term monocultures are not always conducive to maintaining microbial diversity and that this may increase the prevalence of some diseases and reduce yields. Plants (as a source of food, fibre and increasingly biofuels) are subject to huge number of pathogen and predator attacks as well as a range of abiotic stresses. The details of every stage of the host-plant pathogen relationship in a wide variety of soils and a changing climate is a necessary precursor for developing commercial inoculants for biocontrol (Fravel 2005).

The turnover of organic C in soil is a major part of the global carbon cycle and has a large impact on atmospheric CO_2. The carbon dioxide arises from root respiration and the mineralisation of rhizodeposits, as well as the degradation of the generally more recalcitrant humic-like organic matter. Increasing temperatures will give rise to a greater rate of microbial metabolism (just think microbial enzymes and temperature optima) and a consequent reduction in the soil organic carbon sink—accompanied by yet more respired carbon dioxide.

Plants and their rhizosphere microorganisms have a love–hate relationship that is forever being reassessed and modified. These interactions have co-evolved and will continue to do so, but our increasing understanding suggests that we may soon be able to influence this process and use it to our advantage.

Some of these interactions will prove critical for the successful development of agriculture, forestry and horticulture in the next 50 years, both on our planet and probably beyond (Table 2).

Table 2. Manipulating the rhizosphere in the 21st century

- Using microbial inoculants or manipulating the indigenous population for rational biocontrol and bioremediation
- Controlling the release of plant nutrients and the enhancement of other plant growth promoting processes
- Exposing plants to microorganisms (or microbial metabolites) to induce a specific or general immune response to pathogens and predators
- Planting crops that are compatible with the indigenous microflora, soil type and climate
- Stimulating or designing rhizospheres that can support plant growth in a changing climate and in soils that are stressed by salinity, pollution, erosion and low available nutrients
- Selecting or designing bacteria and fungi with rhizosphere 'competence'
- Breeding plants that constitutively or inductively secrete signals that generate and support a beneficial rhizosphere
- Designing self-sustaining soil biospheres for agricultural production in crowded cities and, eventually, for establishment on the Moon, Mars and beyond

The synthesis and secretion of chemical signals by plants and microorganisms are reciprocal, tightly regulated and controlled, and are both caused by and initiate a cascade of events. We don't know what we don't know but it is likely that there are many more interactions taking place in the rhizosphere than we suspect—but they are certain to be intricate and somewhat resistant to understanding and ultimately manipulating. We can be certain that our desire for a simple, consistent and generic resolution will be dampened: Occam's razor will be blunted when attempting to understand the intermittent and often chaotic processes that occur in the rhizosphere. The established scientific belief in reductionism, that posits that everything in the natural world can be understood through a full knowledge of its constituent parts, may not hold true for all of soil biology.

Conclusions

The signposts erected by Albert Rovira and his many colleagues and his continuing enthusiasm and involvement (e.g., Ryder *et al*. 2007; Gupta *et al*. 2010) give us direction and optimism about the future. Plant–microbe–soil interactions are a big part of that future. In the undeniably important context of food production and environmental quality, it could be argued that understanding the multitude of relationships within the rhizosphere is the most challenging and important aspect of 21st century agricultural research. Some of the science is already in place or there is a sufficient momentum that ensures that many of the answers will be forthcoming. What we need now is the political will and financial commitment to make further breakthroughs and convert our knowledge into practice. Future discoveries will be driven by sound and imaginative investment and sophisticated technologies, and limited only by the imagination of the researcher. An attitude that nothing is impossible and a refusal to be daunted by the apparently bottomless black box of soil will lead to some startling discoveries and some highly successful agricultural and environmental applications over the next decade.

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How best can we design rhizosphere plant–microbe interactions for the benefit of plant growth?

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Abstract

The rhizosphere represents a dynamic front or region where plants meet and interact with beneficial and pathogenic microbes, invertebrates, other plant roots and soil. Since the identification, in the early 1950s, of involvement of root exudates in the plant–microbe interaction a lot has become known about the composition of the compounds and the complex nature of the rhizodeposits involved, their possible role in controlling specific plant–biota interactions and soil, and plant and environmental factors that affect the process. However, our ability to specifically manipulate these interactions for the benefit of plant health and growth is yet to successfully and reliably reach the field environment. Examples of root exudate or rhizodeposition induced changes in plant–microbe interactions include (i) proliferation of specific genera or groups of biota, (ii) induction of genes involved in symbiosis and virulence, (iii) promoter activity effects in biocontrol agents and (iv) genes correlated with root adhesion. The observation of plant variety-based differences in root exudation/rhizodeposition and associated changes in rhizosphere microbial diversity suggests the possibility for the development of varieties with specific root–microbe interactions for improved field performance. The effect of foliar sprays of herbicides and nutrients on root exudation and microbial communities opens the possibility of managing rhizosphere biological activity from above ground, that is a 'designer rhizosphere' to suit edaphic and environmental variation. Although new knowledge about the enormous diversity of the soil microbial genome and the nature of chemical language that can occur suggests a vast complexity to the potential interactions, recent developments in techniques provide hope for the identification of critical factors to help manage this dynamic front for the benefit of the plant.

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Introduction

The importance of plant–microbe interactions in the rhizosphere region has been recognised and appreciated for more than 50 years (Rovira 1956; Lynch 1990; Drinkwater and Snapp 2007; Hawkes *et al.* 2007). A number of reviews and books detail the various aspects of the rhizosphere from both the plant and microbial perspective (Bowen and Rovira 1999; Singh *et al.* 2004; Watt *et al.* 2006; Cardon and Whitbeck 2007). We now know a lot about the spatial and temporal dynamics of different microbial groups in the rhizosphere and have some knowledge of the factors that influence the populations and activities of soil biota in the rhizosphere.

Through rhizodeposition plants can affect rhizosphere microbial diversity and activity and resistance to pests and diseases; support benefi-

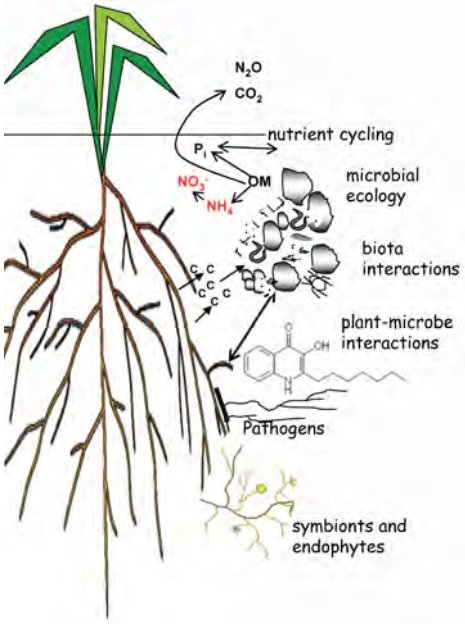

Figure 1. Root exudate or rhizodeposition mediated plant–microbe–soil interactions with impacts on plant growth and productivity (Elizabeth Drew and V.V.S.R. Gupta, CSIRO, unpublished)

cial symbioses; affect nutrient cycling and alter the chemical and physical properties of soil and allelopathy against competing plants (Fig. 1). Root–microbe, root–biota and root–insect interactions can be grouped as beneficial, deleterious or symbiotic associations in relation to nutrient cycling, plant health and growth. Compounds in root exudates and border cells have been shown to act as chemo-attractants or suppressors influencing population levels of rhizosphere biota including bacteria, fungi and nematodes (Bowen and Rovira 1999). Root-exudate-induced gene expression in legume–*Rhizobium* symbiosis has been well studied. However, root-exudate-induced gene expression in non-obligate plant–microbe relationships is less well understood. For example, it may be possible to construct organisms with rhizosphere-controlled beneficial genes and to use them as gene delivery vehicles for specific purposes. A number of soil, plant and environmental factors and management practices can affect the quantity and quality of rhizodeposition that in turn affect the composition and activity of rhizosphere biota communities. Soil physical structure can affect the rate of root growth and structure, which influences the quantity of root exudation, whereas chemical properties can interfere with or enhance the effectiveness of chemical signals by plants and microbes. In this paper we review, briefly, what is known about plant–microbe interactions and provide examples where rhizosphere microbial populations have been influenced through above-ground management.

Link between plant and rhizosphere microbial communities

The key link between a plant and the microbial community is the release of a diverse range of root exudates and rhizodeposits by the plants below-ground. A plethora of organic compounds have been identified in root exudates and rhizodeposits, for example root-specific chemical signals, low and high molecular weight carbon and nutritional compounds, growth regulators and secondary metabolites (Lynch 1990; Bowen and Rovira 1999; Bais *et al.* 2006; Badri and Vivanco 2009). Root exudation is the major source of organic C released by plant roots. Whipps (1990) reported that young seedlings exude as much as 30–40% of their fixed carbon. The quantity and quality of root exudates are determined by the plant species and the age of the plant modulated by the external abiotic and biotic stress factors (Rovira 1965). Although rhizodeposition is sometimes considered as a burden to the plant through loss of photosynthetic product, it is now established that plants use this physiological process to benefit from the rich diversity of soil microorganisms. From the soil biota perspective, the rhizosphere is a niche for large microbial populations and heightened activity, in particular in low-organic-matter (i.e. oligotrophic) agricultural soils. Plants can affect the composition and/or activity of beneficial and deleterious microbial communities and thus alter the complex trophic interactions and relations between plant, microorganisms and soil fauna. Larger populations of microflora in the rhizosphere encourage the predator–prey interactions that are important in stimulating plant growth both through the 'microbial loop in soil' and a number of significant indirect feed-backs between consumers of rhizosphere microorganism and plant roots (Bonkowski *et al.* 2009).

The rhizosphere is a dynamic changing environment differing throughout the plant growth period. The rhizosphere effect is considered to extend only a few millimetres from the root, but its extent will depend

upon the diffusivity of particular root exudates as influenced by soil characteristics and the water status of soil. Spatial differences in rhizosphere microbial communities can also be seen within the root system from the root tip to behind the zone of elongation (Foster 1986; Bowen and Rovira 1999), with the maximum rhizosphere effect postulated to be in the region behind the zone of elongation. The composition and activity of rhizosphere microbiota (microflora and protozoa) not only varies between plant species, but intraspecific (e.g. variety-level) variation within a plant type has also been demonstrated. For example, *Brassica* plants support a significantly different composition of fungal and bacterial communities compared with cereal plants, while different canola varieties also differ in their rhizosphere bacterial populations (Siciliano and Germida 1998; Gupta *et al.* 2009; Hartmann *et al.* 2009). Plant effects can also be modified by the nutrient status of the plant, by mycorrhizal colonisation and by pathogen infection.

Factors that affect root exudation or rhizodeposition

A large body of evidence exists indicating the influence of diverse physical, chemical and biological factors on root exudation and/or rhizosphere microbial populations (Bowen and Rovira 1999; Cardon and Gage 2006). In addition the root system architecture, a plant trait determined by its genetic predisposition and subsequently modified by several biotic and abiotic factors can alter exudation characteristics (Badri and Vivanco 2009). In seminal papers published during 1956, Rovira *et al.* described the composition of root exudates and demonstrated the link between plant species, exudate compounds and changes in specific microbial populations. Despite more than 50 years of subsequent research, the underlying genetics for the differences in plant response to factors affecting root exudation and rhizodeposition remain unclear.

As plant roots in soil are exposed to a variety of abiotic and biotic stresses, they release a complex blend of chemicals to encourage positive interactions and protect against negative influences. The effects of soil, environment, plant and management factors on root exudation have been reviewed by Rovira (1969), Cardon and Whitbeck (2007) and Badri and Vivanco (2009). These can be briefly summarised as:

- The quantity and composition of root exudates varies depending upon the type, age and physiological status of the plant.
- Soil physical structural properties that cause mechanical impedance affecting root morphology, for example high bulk density and soil compaction, can increase root exudation.
- Soil chemical characteristics including pH, nutrient status and the presence of particular minerals and toxic metals can alter the quantity and composition of root exudates. For example, acid soil pH increases organic anion malate exudation. Similarly plant roots have been shown to exude specific organic acids (e.g. citric, oxalic and malic acids) to detoxify high levels of aluminium in soils (Badri and Vivanco 2009). Phosphorus-deficient plants develop greater amounts of fine roots and can secrete phenolic compounds and specific organic anions. Higher salt concentrations in soil increase the amount of root exudation.
- Environmental factors such as temperature, light and rainfall can modulate root exudation or rhizodeposition processes; for example Watt and Evans (1999) reported that the root exudation process follows diurnal rhythms, with higher exudation during periods of light. Rainfall and soil moisture conditions affect root exudation through water stress or oxygen concentrations in soil.
- Exposure of plants to agrochemicals (e.g. insecticides, herbicides and fungicides), hormones and nutrients can alter both the quantity and composition of root exudation.
- The presence of specific microorganisms or microbial metabolites can alter the secretion of root exudates. For example, Meharg and Killham (1995) reported that microbial inoculation of the rhizosphere may increase root exudation by a number of modes of action—that is, beneficial metabolites increase exudation through stimulation of root growth—whereas fungal infection of plants can alter the composition of root exudate.

Response of specific functional groups of microbes, populations and function

Soil microorganisms utilise their capabilities for chemotaxis, induced gene expression and competitiveness to colonise roots and grow in the rhizosphere. Some examples of root exudate mediated plant–microbe interactions are listed in Table 1. Most rhizosphere microorganisms, being heterotrophic, depend on rhizodeposits as carbon and nutrient substrates. Rhizosphere microorganisms also use minor, non-nutrient components in root exudates as signals to guide their movement towards the root surface and elicit changes in gene expression appropriate to the environment. Examples of such interactions involving symbiotic rhizobia and mycorrhizae have been extensively investigated (Bauer and Caetanoanolles 1990; Long 2001; Bais *et al.* 2006). Similarly certain compounds in root exudates, even at low concentrations, can serve as chemo-attractants for rhizosphere bacteria. For example, the ability of *Azospirillum* spp. to detect low concentrations of aromatic compounds such as benzoate confers an advantage in its survival and colonisation of rhizospheres (Lopez-de-Victoria and Lovell 1993). In addition to attracting specific microorganisms, components or root exudates can serve as specific inducers of gene expression, for example virulence and catabolic genes, and promoter activity for specific genes. van Overbeek and van Elsas (1995) reported that root exudates such as proline induced specific promoter activity in plant-growth-promoting *P. fluorescens* in the wheat rhizosphere. Burgmann *et al.* (2005) found specific components of root exudates selectively altered the structure and activity of soil diazotrophic communities—for example, sugar-containing substrates were able to induce nitrogen fixation—but the presence of organic acids and substrate concentration may have additional effects on the active diazotrophic population. Exudation of antimicrobial metabolites has been suggested as a major mechanism for plants to withstand pathogen attacks (Hartmann *et al.* 2009). The ability of plants to produce antimicrobials, such as antioxidant enzymes, necessitates the requirement for successful rhizosphere colonisers to avoid these chemicals.

Table 1. Some examples of the root-exudate-induced changes in plant–microbe interactions

- Induction of genes involved in symbiosis and virulence (Downie 2010)
- Proliferation of specific genera and/or groups of biota
  - *nif*H phylotypes (Bürgmann *et al.* 2005)
  - Suppressive bacteria (Smith *et al.* 1999)
  - Plant beneficial pseudomonads (de Weger *et al.* 1995)
- Promoter activity in PGPR / biocontrol agents (van Overbeek and van Elsas 1995; Kloepper *et al.* 2004)
- Gene expression related to root adhesion (Downie 2010)
- Genes associated with border cell quantity and quality (Hawes *et al.* 1998)

Border cells

One of the major components of rhizodeposition is the border cells, originally called 'sloughed root cap' cells, which are enmeshed in the mucilage but become detached in the presence of free water (Hawes *et al.* 1998). It is suggested that border cells act as a 'front line' of detached living cells that help the parent plant cope with attack by an array of soilborne organisms awakened to growing root signals. They are living cells that can survive as long as appropriate nutrients are available and they are protected from predators and other stresses (Hawes *et al.* 1998).

Diverse border-cell-mediated responses in soil organisms—both beneficial and pathogenic (e.g. bacteria, fungi and nematodes)—have been reported (Hawes *et al.* 1998). They include the general stimulation of growth and sporulation of bacteria and fungi, chemoattraction or repulsion of beneficial and pathogenic organisms, and production of specific extracellular signals that influence microbial gene expression. Evidence also exists for border cell production of extracellular enzymes, antibiotics, phytoalexins and a number of unknown proteins of functional significance. It is also proposed that border cells may act as a decoy for pathogens (fungi and nematodes) before they can reach the growing root tip and cause infection.

Although border cells are sometimes seen as just a source of nutrients for invading microorganisms, a

number of studies have reported host- and tissue-specific responses of border cells to microorganisms: that is, the ability of any given microorganism to associate with border cells may depend upon the plant genotype (Hawes and Brigham 1992). Knox *et al.* (2007) reported varietal-based differences in the number of border cells produced by cotton seedlings. In general, border cell production differed considerably among cotton cultivars, but border cell production in the transgenic cultivars of cotton (e.g. cultivars expressing *Cry*1Ac and *Cry*2Ab) was similar to that of both donor and elite parents. However, one of the parental cultivars, Sicot 189, produced substantially greater numbers of border cells compared to all its transgenic derivatives. A comparison of border cell number with varietal disease resistance ranking against *Fusarium* wilt of cotton found a limited relationship ($r^2 = 0.65$).

Border cell production is not generally a continuous by-product of constitutive turnover of the root cap, but can be turned on and off by the plant. In addition, border cell production can be influenced by environmental conditions. Zhao *et al.* (2006) observed that CO_2: O_2 concentrations in the soil atmosphere can influence the development and separation of border cells in pea seedlings, but no such effect was seen in alfalfa.

The nature of the interaction between border cells and specific members of the soil microbial community is thought to be regulated by (i) the carbon and nutrient composition of border cells or (ii) defense structures or attractants produced by border cells or (iii) the nature of the mucigel. For example, zoospores of *Pythium dissotocum* accumulate on border cells of cotton (Goldberg *et al.* 1989) whereas border cells of single maize can synthesise defence structures that repel penetration by *Colletotrichum graminicola* hyphae (Hawes *et al.* 1998). Such specific associations, combined with designed border cell production and release, could be used to improve plant nutrition (e.g. through border cells producing nutrient-solubilising enzymes), impart disease resistance (e.g. border cells delivering antibiotics to evade pathogen infection) and help with bioremediation.

Quorum-sensing communication

Quorum-sensing (QS) behaviour refers to the population-density-dependent regulation of gene expression in bacteria. It works by the exchange of signal molecules between nearby cells that enables individual cells or small colonies of bacteria to coordinate the expression of specific genes and thereby act like a multicellular organism. Plants have been shown to release compounds that mimic bacterial signals and take advantage of bacterial dependence on QS (Bauer and Mathesius 2004; Juhas *et al.* 2005) and produce enzymes that degrade quorum-sensing compounds and disrupt microbial communication (quorum quenching) before the population reaches the critical level required to be effective. This phenomenon has been shown to exist both in beneficial and pathogenic bacteria and fungi (Fuqua *et al.* 1994; Nickerson *et al.* 2006; Cooper 2007). N-acyl homoserine lactones (AHLs) in gram-negative bacteria and small peptides in gram-positive bacteria are the commonly reported QS signals. In addition to the evidence for QS signal production by diverse bacterial genera, disruption of QS regulation by other bacteria (i.e. signal degraders) adds additional complexity to plant–bacterial communication. The bacterial responses to QS signals include altered biofilm dynamics, motility, plasmid transfer, stress response, virulence, nitrification and enzyme production (Pierson *et al.* 1998, Bauer and Mathesius 2004; d'Angelo-Picard *et al.* 2005). Examples of plant responses to bacteria QS signals are defence-related gene expression, stress responses and expression of symbiosis genes. This research is currently in its infancy; before we can expect to manage plant responses through the regulation of QS signals we require a better understanding of their production and degradation, community-level responses and the diverse array of traits that respond to QS communication. Bauer and Mathesius (2004) suggested that genome-level transcriptional studies are needed to gain a better understanding of the dynamics of QS signals and their influence on plant-related functions.

Effect of inoculation with micro-organisms on rhizosphere microbial communities

As indicated before, the presence of specific microorganisms in the rhizosphere can modify the quality and quantity of root exudation. In recent times, a great deal of research has been done to inoculate seeds with bacterial, actinomycete and fungal isolates for the purpose of bio-protection against biotic stresses including pathogens or

plant growth promotion (Kloepper *et al.* 2004; Raaijmakers *et al.* 2009). A number of these organisms are known to produce biologically active compounds, for example hormones, antifungal compounds and other types of antibiotics that have the potential to modify the composition of rhizosphere microbial communities. In general, transient changes in indigenous bacterial, fungal and protozoan populations, carbon turnover and enzyme activities have been observed in response to inoculation of plants with specific organisms, but many of these effects are based on laboratory experiments and information from field environments is limited. Natsch *et al.* (1997) reported that inoculation with antibiotic-producing *P. fluorescens* inoculants resulted in only a transient reduction in resident pseudomonads on wheat roots. Glandorf *et al.* (2001) found that application of genetically modified phenazine (PCA)-producing *P. putida* (engineered to have improved antifungal activity) exerted non-target effects on the composition of natural fungal microflora in the wheat rhizosphere when measured using 18S rDNA ARDRA analysis. They found that PCA-producing genetically modified (GM) plants can transiently alter the composition of rhizosphere fungal populations of field-grown wheat. However, the inoculants had no effect on metabolic activity and biological processes such as nitrification potential and cellulose decomposition. Robleto *et al.* (1998) showed that the inoculation of field-grown *Phaseolus vulgaris* with *Rhizobium etli* strains, producing peptide antibiotic trifolitoxin, caused a reduction in the diversity of trifolitoxin-sensitive α-proteobacterial populations. These studies suggest that it may be possible to alter rhizosphere microbial communities, at least transiently, to a level that affects plant growth.

Over the last two decades, there is a growing interest in exploiting rhizosphere- or endophytic-colonising non-pathogenic bacteria to increase plant resistance to abiotic stresses such as salinity, drought and metal or contaminant toxicity (Dimpka *et al.* 2009). For example, it has been suggested that osmolytes such as glycine betaine produced by osmo-tolerant bacteria could potentially act synergistically with plant-produced glycine betaine in response to stress and thus increase drought tolerance by plants (Dimpka *et al.* 2009). The plant growth promoting rhizobacteria (PGPR) effects of bacteria possessing 1-aminocyclopropane-1-carboxylate (ACC) deaminase have been shown to help plants withstand both abiotic and biotic stresses (Hontzeas *et al.* 2004). Some of the known mechanisms regarding root–microbe–soil interactions when exposed to abiotic and biotic stressors include:

- Bacterially produced indole acetic acid (IAA) or nitric oxide stimulating root growth under drought, salinity and metal toxicity and nutrient deficiencies
- Bacterial ACC deaminase reducing host plant stress ethylene level
- Changes in plant physiological and phenotypic characteristics, for example cell membrane.

Since the occurrence of multiple stresses is not uncommon in field environments, it would be beneficial to identify microbial inoculants that can provide cross-protection against both biotic and abiotic stressors, to sustain agricultural production in response to climate change.

Modification of rhizosphere composition through above-ground treatment

In high-input agricultural systems, the needs of the plant are generally met by external applications of nutrients and other agrochemicals, but in low-input agro-ecosystems, especially on soils with lower organic matter, there is a need to broaden the rhizosphere contributions to encompass a wide range of plant-essential biological processes in order to maximise crop growth and yields. Until now most of the deliberate management of rhizosphere plant–microbe interactions has focused on the control of plant pathogens and improvement of symbiotic associations such as nitrogen fixation and mycorrhizae. The effect of management practices on other rhizosphere microbial processes has been researched for over 50 years, but efforts to modify these processes to benefit crop growth and production have been inconsistent or ineffective (Handelsman and Stabb 1996; Rovira 1991).

Here we discuss, briefly, examples of changes in rhizosphere microbial diversity and/or functions due to genetic variation (e.g. crop or variety choice), application of chemicals or nutrients as part of crop management.

Plant variety-based differences in plant–microbe interactions

Plant genotype-based differences in rhizosphere and endophytic microbiota populations have been well demonstrated for a variety of plant types. Neal *et al.* (1973) reported that even a single chromosome substitution in the plant genotype has the potential to alter the composition of microbial communities. The role of plant species, and inter-specific and intra-specific variation, in determining rhizosphere microbial communities in an agricultural context was reviewed by Drinkwaer and Snapp (2007) and Hawkes *et al.* (2007).

Until recently plant breeding programs have generally intended to improve above-ground characteristics and agronomic fitness of plants, even though such modifications could alter below-ground plant parts—that is, root growth pattern and rhizodeposition, and root-associated microbial community and biological functions. Reported differences in root architecture (distribution in depth, morphological features) and exudate quality for wheat cultivars have been linked to tolerance of moisture and salt stresses, heavy metal contamination, root disease avoidance and improved nutrient use efficiency.

During the last decade, in a series of glasshouse- and field-based research projects, we explored the relationship and interaction between genetically modified and conventional cotton varieties with their associated rhizosphere microbiology. Results indicated that cotton rhizosphere plant–microbe interactions are significantly influenced by cultivar type, irrespective of their GM status (Gupta *et al.* 1998; Knox *et al.* 2004, 2009). A very strong relationship was observed from the start of the season between the bacterial communities that develop in the rhizosphere of cotton varieties. Although there were seasonally-based differences in the populations of rhizosphere microbial communities, a clear varietal separation was observed. Differences in the genetic and catabolic diversity of microorganisms between varieties suggest that rhizosphere microbial communities may be adapted to the quantity and quality of root exudates from cotton plants.

Germida and Siciliano (2001) reported that there was significant variation between the bacteria generally associated with modern and older wheat cultivars. For example, bacterial endophyte communities of more modern wheat cultivars were more diverse than those of ancient land races, and were aggressively colonised by endophytic pseudomonads. Siciliano *et al.* (1998) suggested that the root morphologies and chemical composition of modern cultivars affected the ability of certain rhizosphere bacteria to colonise the root interior. Gupta *et al.* (2009) reported that populations and/or composition of a number of phenotypic and functional groups of microflora—for example copiotrophic and oligotrophic bacteria, cellulotyic microflora, total pseudomonads, spore-forming bacteria and nitrifying bacteria—differed in the rhizosphere soils of different wheat varieties. Wheat varieties also differed in terms of rhizosphere protozoan composition (Fig. 2). Community-level physiological profiling analyses indicated variety-based differences in bacterial composition

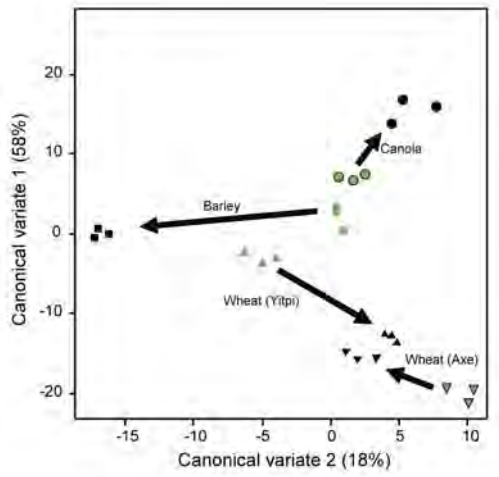

Figure 2. Influence of foliar herbicide exposure of plants on the catabolic diversity of soil microbial communities in the rhizosphere of canola, barley and varieties of wheat. Data on the carbon substrate utilisation profiles were obtained using a modified Microresp® method (V.V.S.R. Gupta, 2007, unpublished) with rhizosphere soils collected 5 days after the foliar application of the herbicide Sprayseed® (equivalent of 0.081 g of Diquat and 0.069 g of Paraquat in 1 L of distilled water) on 4-week-old seedlings in a glasshouse experiment. Canonical variate analysis was conducted on the CO_2 data normalised against the average response for 31 different carbon substrates. Green-filled symbols are for control plants and black-filled symbols are for herbicide-exposed plants.

(Sicliano *et al.* 1998; Gupta *et al.* 2004). Since the effect of the plant on the soil microbial community is primarily through the changes in rhizodeposition, catabolic profiling can provide links to plant influences on rhizosphere microbial communities. Such differences in microbial genetic and catabolic diversity and abundance between varieties can be reflected in the biological processes involved in C and N cycling (Gupta *et al.* 2009; Knox *et al.* 2009).

Gupta *et al.* (2004) reported that wheat cultivars differentially select for different microbial communities that can affect the growth and productivity of the following wheat crop. Wheat-following-wheat in field trials can result in a 5–20% yield penalty due to a build-up of deleterious microflora, and there is a strong effect of variety. For example, larger populations of fast-growing bacteria (copiotrophs) were associated with poor-performing varieties, while larger populations of slow-growing bacteria (oligotrophs) were associated with high-performing varieties. In addition, wheat cultivars as seedlings differ in their ability to select for a disease-suppressive microbial community in wheat-soil bioassays (Dr S. Barnett, SARDI, pers. comm., 2008).

A plant's influence on the root-associated microbiota (e.g. microflora and protozoa) can vary at different microsites along the root, and it can change during the growing season. In addition, the effect of the plant on rhizosphere communities is modulated by the soil type in which the plant grows and by the nutritional status of the system. For example, endophytic bacterial and actinomycete communities in the roots of crops are a subset of the rhizosphere community (Siciliano *et al.* 1998; Conn and Franco 2004). Guemouri-Athmani *et al.* (2000) found that the genetic composition of *Pseudomonas polymyxa* populations varied across the chronosequence in Algerian soils that had been under wheat cultivation for from 5 to 2000 years. The longevity of wheat cultivation was correlated with decreased genetic and phenotypic diversity and higher frequency of N-fixing strains of *P. polymyxa* in rhizosphere soils. Schallmach *et al.* (2000) found that low N status increased the proportion of α-proteobacteria relative to the entire bacterial populations near the root-tip of common bean. Briones *et al.* (2002) reported that links between plant genotypic and phenotypic differences in rhizosphere processes occur in nitrifying bacterial populations in the rhizosphere of traditional versus modern rice cultivars.

Overall, these observations suggest that further understanding of mechanisms governing rhizosphere plant–microbe interactions will be crucial in the development of crop-breeding strategies that target the selection of plant–microbe consortia or a 'designer rhizosphere' approach for the benefit of agriculture in the future. A number of edaphic, environmental and management-related factors have been shown to influence the complex association between plant roots and soil microbial communities—both the numbers and types of organisms and their functionality. The manipulation of plant–microbe interactions can be achieved by (i) altering rhizodeposition through variety or crop choice, and/or (ii) modifying rhizosphere microbial diversity and functionality through management, that is tillage, rotation or application of chemicals.

Effect of above-ground treatment of plants on rhizosphere communities

A variety of management practices involve the above-ground treatment of plants with agrochemicals, hormones and nutrients to either control pests and diseases or help improve plant nutrient status. Such treatments generally improve plant health, but sometimes may cause short-term or transient invigoration or stress in the plant. A number of studies have reported increased severity of root diseases following the exposure of plants to herbicides, even at low levels (Bowen and Rovira 1999), and such observations have been attributed to the increased susceptibility through increased and/or altered root exudation influencing root–pathogen–microbe interactions. In addition, aerial sprays of pesticides or nutrients have been shown to influence the rhizosphere microflora of a number of crops (Agnihotri 1964; Rovira 1965). Aerial sprays of plants with urea were shown to modify bacterial and fungal populations in the rhizosphere of a variety of crops such as wheat, maize and tea (Rovira 1965), and these changes were attributed to modifications in the quality and quantities of root exudates. Other examples of foliar-spray-mediated modification to rhizosphere microflora occur with antibiotics and hormones in the sprays; results have been variable.

The introduction of herbicide-tolerant (HT) crops has changed the timing and spectrum of herbicides used 'in crop' and provided farmers with an effective in-crop weed-management tool. For example, glyphosate-resistant varieties of crops such as canola,

soybean and wheat have been quickly and widely adopted (Beckie *et al.* 2006). HT crops change soil environmental conditions and can influence microbial communities through (i) direct effects of herbicides on microbes, (ii) introduction of plant material (dying weeds) from post-emergent herbicide use, and (iii) influencing microbial dynamics in the rhizosphere. The effects of HT crops on soil microbial communities are variable depending upon the herbicide chemistry, plant type and edaphic factors (Hart *et al.* 2009). Recent reports suggest that roots of glyphosate-resistant soybean cultivars treated with glyphosate may be colonised by *Fusarium* sp. (Kremer 2003; Means and Kermer 2007). Kremer *et al.* (2005) reported that glyphosate application increased exudation of carbohydrates and amino acids. In vitro bioassays indicated that the presence of glyphosate and root exudate compounds stimulated growth of selected rhizosphere fungi and reduced populations of bacteria such as pseudomonads. Gupta *et al.* (1998) reported that application of herbicide pyrithiobac-Na increased the level of microbial activity (by over 40%) and altered the catabolic diversity of bacterial communities in the rhizosphere of GM cotton, but had only a slight effect in the parent cotton. Research from Canada clearly demonstrated differences in the endophytic and rhizosphere communities of transgenic and non-transgenic canola varieties grown at the same field site (Siciliano *et al.* 1998). Research conducted in Australia has indicated that the genotypic and catabolic diversity of rhizosphere bacterial and fungal communities of herbicide-tolerant and conventional canola varieties differ in their response to in-crop herbicide application (Gupta *et al.* 2009). Yousseff *et al.* (1987) reported that herbicide application to cotton increased amino acids in exudates and that this increase was linked to an increase in fungal populations, and that the effects were soil-type-dependent.

Results depicted in Figure 2 show significant changes in the catabolic diversity of microbial communities when different plants (canola, wheat and barley) are exposed to the same chemical, and that such responses can also vary between varieties of the same crop, for example wheat varieties Yitpi and Axe. Plants express stress symptoms when exposed to unfavourable conditions (including chemical exposure) through altered quantity and quality of rhizodeposition, resulting in modifications to the composition and or functionality of microbial communities. Overall, these examples indicate that it would be possible to manipulate plant–microbe interactions as required through above-ground chemical application. As Bowen and Rovira (1999) suggested, since herbicide exposure can affect both the pathogenic and beneficial plant–microbe interactions it may be possible to direct specific rhizosphere microbes from the above-ground management of crops, that is achieve 'designer rhizospheres' to suit specific edaphic and environmental conditions.

Until now rhizosphere responses have been generally investigated in terms of the release of C and nutrients, whereas mechanisms involving the production and release of other signal molecules that can influence molecular cross-talk between plants and microbes or among rhizosphere microbial communities have not been investigated. New developments in molecular-based methods can now help us unravel the molecular interactions underlying the plant- and microbial-based mechanisms in order to successfully apply ecological knowledge to agricultural management.

What is needed?

Since Lorenz Hiltner coined the term 'rhizosphere' more than hundred years ago, ample evidence has been obtained to demonstrate the selection of microbes by plant roots. However, the complexity of plant–microbe interactions within both the root and the rhizosphere still requires the unravelling of major steering factors that control specific interactions. We need to improve our ability to alter rhizodeposition to selectively stimulate and/or suppress certain groups of microorganisms, leading to new community structures and ultimately improving plant growth and productivity, especially in the field environment.

Our work has added to the body of evidence that identifies that changes are brought about in population size in both phenotypic and functional groups of microbial and microfaunal communities and in their ability to function (e.g. nitrification, free-living N_2- fixation, C turnover) through the selection of varieties and in-crop management practices. However, the science with regard to how we can design rhizosphere plant–microbe interactions for the benefit of plant growth remains in its infancy, and we now need to develop it by addressing some of the outstanding issues, which include, but are not limited to:

- **Signalling molecules that stimulate rhizosphere population growth.** These could be simple sugars or more complex molecules triggering growth responses, such as plant flavonoids, homoserine lactones (HSL), or IAA. What is the benefit of increasing some populations? Can pathogen control be effected through competition or physical barriers? Could increased populations simply attract higher trophic groups (protozoa or nematodes) and thus enhance nutrient cycling?
- **Signalling molecules that stimulate function.** Increased function has been observed where the size of the population involved does not correlate well with activity, which implies a shift in functional capabilities, possibly due to genetic switches or channel-stimulating molecules and signals. When would stimulation of a function without a population change be beneficial? Is functional stimulation better by being more controlled or targeted than population control, thus potentially avoiding proliferation of pathogens or organisms that could benefit neighbours?
- **Below-ground drivers of above-ground diversity.** There is a growing body of work that demonstrates that a more diverse mycorrhizal soil community will support a more diverse plant community. Is it possible that bacterial communities could be having a similar effect on plant diversity, or are they simply too numerous, functionally capable, recalcitrant and resistant to have a role as a driver in this process?

With the current focus on security of food to feed the growing global population and a need to address issues of soil health maintenance, agricultural productivity and greenhouse gas production, we need improved ecosystem strategies that require knowledge of regulators and feedbacks for rhizosphere interactions against background soil environment and function over longer temporal scales (Gupta *et al*. 2010). A better understanding of the dynamics of rhizodeposition in natural and agricultural systems will be needed in order to establish the underlying genetic background for both the production and reception of the signal molecules involved. Expansion of these systems-level processes to a range of plant species and habitats will also be required, with the acceptance that in some cases habitat- and species-specific signal molecules will need to be identified where present.

Finally, the path to achieving a designer rhizosphere will be through the description of the dynamics of microbial composition and defining the factors that regulate their functionality (Burns 2010, pp. 1–10 this volume). In achieving this, key areas of interest include (i) identifying the plant signals and microbial receptors that cause and recognise the changes, (ii) identifying what changes in either population composition, functional activity or both are effected, (iii) identifying why the changes are important within the wider function of evolution and ecology of the production system, and (iv) the scope of the environmental conditions and/or other external factors under which the changes can be effected.

Conclusions

In the last 50 years the science of the rhizosphere and the plant–microbe interactions occurring within this narrow frontier has come a long way. The ability to use management or crop variety selection to effect change in rhizosphere population composition or functional activity has been clearly demonstrated. On the back of this comes clear recognition of the potential to develop methods within existing agricultural systems that could either take advantage of or be managed for beneficial manipulations of the associated microbiota within this dynamic front, that is a 'designer rhizosphere'. The decades ahead will hopefully see many of the challenges we have outlined here addressed and, perhaps in another 50 years, we will no longer have to ask how best to manage the plant–microbe interactions of the rhizosphere for plant growth, because we will already know.

Acknowledgements

Financial support for the authors was provided by CSIRO Divisions of Land and Water and Entomology and through projects funded by Grains and Cotton Research and Development Corporations. Technical support for laboratory analyses was provided by Stasia Kroker.

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Nature of biocontrol of take-all by the Australian bacterial isolate *Pseudomonas* strain AN5: a true symbiosis between plant and microbe

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Abstract

Biological control of the take-all fungal pathogen *Ggt* by *Pseudomonas* bacteria is dependent on its ability to colonise wheat roots and produce an anti-fungal agent. *Pseudomonas* strain AN5 *(Ps.* str. AN5) is a non-fluorescent bacterial isolate from Australia that has been shown to suppress take-all disease and increase wheat yield by up to 20% in field trials. *Ps.* str. AN5 has been developed as a commercial biocontrol agent. *Pseudomonas* fluorescence biocontrol bacteria producing the anti-fungal agents 2,4-diacetylphloroglucinol or phenazine-1-carboxylic acid, which suppress the take-all pathogen, have been well characterised previously. *Ps.* str. AN5 does not produce these anti-fungal agents. It produces the sugar acid D-gluconic acid in suppression of the take-all pathogen. We have identified, cloned and sequenced eight regions in the *Ps.* str. AN5 genome responsible for biocontrol.

We have shown *Ps.* str. AN5 wheat root colonisation is a specific process in which the *Pseudomonas* induces erosion zones leading to the bacteria colonising the epidermal and cortical regions. Transposon mutants involved in biocontrol have been identified and characterised. We have developed TaqMan assays for bacterial genes involved in biocontrol such as root colonisation and gluconic acid production. Using Affymetrix genechip microarray technology we have identified wheat genes up- and down- regulated by *Ps.* str. AN5 symbiosis with wheat roots. From this we have developed TaqMan assays for wheat genes involved in this symbiosis. The coordinated expression of plant and bacterial genes strongly suggests that this interaction is symbiotic in nature.

Introduction

Biological control of the take-all fungal pathogen *Gaeumannomyces graminis* var. *tritici (Ggt)* by *Pseudomonas* bacteria is dependent on its ability to colonise wheat roots and produce an anti-fungal agent (Haas and Keel 2003). *Pseudomonas fluorescens* biocontrol bacteria isolated in Europe and the US can produce the anti-fungal agent 2,4-diacetylphloroglucinol or phenazine-1-carboxylic acid, which inhibits *Ggt,* the take-all pathogen (Weller *et al.* 2007). The genes involved in production of these anti-fungal agents and their regulation are well understood (Haas and Keel 2003). The Australian continent contains unique soil microbes as it has been geographically isolated. *Pseudomonas* strain AN5 *(Ps.* str. AN5) is a non-fluorescent bacterial soil isolate from New South Wales in Australia (Nayudu *et al.* 1994a).

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Ps. str. AN5 was shown to produce a radically different type of anti-fungal agent (i.e. gluconic acid) against *Ggt* (Kaur *et al.* 2006). Gluconic acid is the first anti-fungal agent to be identified which is a simple sugar and hydrophilic in nature, compared to the hydrophobic benzene-based anti-fungal compounds discovered previously. Using molecular genetic analysis (Nayudu *et al.* 1994b) we have elucidated the nature of take-all biocontrol by this bacteria. The role of *Ps.* str. AN5 genes involved in biocontrol of the take-all pathogen will be outlined in this paper.

The effective control of soil-borne fungal pathogens such as *Ggt* by antagonistic micro-organisms offers an alternative to the use of chemicals in agriculture. Previously *Ggt* and species of *Pseudomonas*, *Bacillus*, *Trichoderma* and Actinomycetes have been isolated from Australian soils and studied for biological control of the take-all disease (Weller *et al.* 2002). Recently the Australian isolate *Pseudomonas* strain AN5 (*Ps.* str. AN5) has been shown to have a completely new mechanism in inhibiting *Ggt,* in that it produces the sugar gluconic acid as an anti-fungal agent (Kaur *et al.* 2006). In this paper we report the ability of *Ps.* str. AN 5 to suppress the take-all pathogen in vitro and in vivo.

Root colonisation is essential in *Pseudomonas* forming a symbiotic association with wheat. Chemotaxis towards the wheat root, attachment to the rhizoplane and utilisation of carbon sources present in the root exudate (rhizosphere competence) have been identified as essential components in this process (Lugtenberg *et al.* 2001). Global regulators have been identified in *Pseudomonas* that are involved in controlling this process (Lugtenberg *et al.* 2001). Quorum sensing at high cell density, which leads to higher levels of expression of some *Pseudomonas* genes, has also been implicated in this process (Wei *et al.* 2006). The infection process of *Rhizobium* bacteria in root nodule nitrogen-fixing symbiosis has been clearly defined (Bender *et al.* 1987). The process by which *Pseudomonas* bacteria interact with wheat roots, however, is still poorly understood. In this paper we describe the precise mode by which *Pseudomonas* strain AN5 (*Ps.* str. AN5) colonises wheat roots and identify transposon mutants deficient in this process.

Rhizobium bacteria induce a visual response on the plant host and as such the symbiotic interaction between this bacteria and its legume host can be well defined. However, the interaction between *Pseudomonas* biological control bacteria and its wheat host has always been considered transient. In this paper we show that *Pseudomonas* bacteria actually invade and live inside the wheat roots, and using Affymetrix genechip microarray technology we show this leads to differential expression of certain wheat genes. In conclusion—for the first time—evidence is presented to show the biological rhizosphere interaction between pseudomonads and wheat roots is a well-defined process and could be considered as a 'plant–microbe' symbiosis.

Results

Biocontrol

Pseudomonas str. AN5 inhibits take-all hyphal growth by 60–70% in agar plate bioassays. It consistently suppresses take-all disease in pot trials. Take-all disease symptoms are reduced significantly on wheat roots from 2.5 (on a scale where 0 is no disease and 5 is maximum disease observed) for *Ps.* str. AN5 treatment with take-all added compared to 4.5 for no bacterial treatment with take-all added. ANOVA of total plant biomass at the end of the experiment showed there were significant differences between treatments. *Ps.* str. AN5 treatment with take-all added was significantly better than the negative take-all treatment control with no bacteria (about a 100% increase). In field trials there was a significant decrease of take-all diseases symptoms in wheat roots and in the crown with *Ps.* str. AN5 compared to untreated controls. In cases where there was significant take-all disease in the field, wheat yield increases of 10–20% were observed with *Ps.* str. AN5. *Ps.* str. AN5 was shown to be a good coloniser of wheat roots (about 10^5 g^{-1} of wheat root) in different soil types but there was no strong correlation with any environmental parameters such as soil moisture. Importantly it was able to survive well at all field trial sites even under very low soil moisture of less than 10%v/v (Fig. 1).

Anti-fungal agent production

Pseudomonas str. AN5 is able to produce the corresponding aldonic acid when provided with aldose sugar galactose or mannose (Fig. 2). However, only gluconic acid production was detected on wheat roots, showing it is the only sugar acid produced by *Ps.* str. AN5 involved in take-all biocontrol.

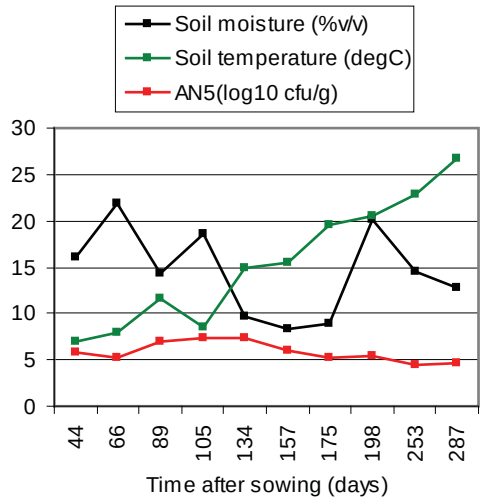

Figure 1. Environmental conditions and survival of *Ps.* str. AN5 on wheat roots at a field trial in Galong, NSW

Pseudomonas str. AN5 produces about 5.0 mg ml^{-1} gluconic acid when glucose is provided as the sole carbon source. This is significantly more than the microgram levels of other anti-fungal agents produced by other *Pseudomonas* bacteria. Using transposon mutagenesis we identified two regions in the *Ps.* str. AN5 genome essential for sugar acid production. Mutants in both these regions completely lost take-all biocontrol in pot trials. Complementation studies using genes or gene products restored take-all biocontrol. The genes present in these regions were sub-cloned from cosmid vectors and their DNA sequence determined. In one region the glucose oxidase gene (GOD) was identified (Nayudu *et al.* 2008b). In another region, six genes required for the production of the cofactor pyrroloquinoline quinone (PQQ) were identified in an operonic structure (Nayudu *et al.* 2008b). Using RT-PCR we have shown these seven *Ps.* str. AN5 biocontrol genes are expressed on wheat roots in the field, suggesting that they are involved in take-all biocontrol (Nayudu *et al.* 2008a).

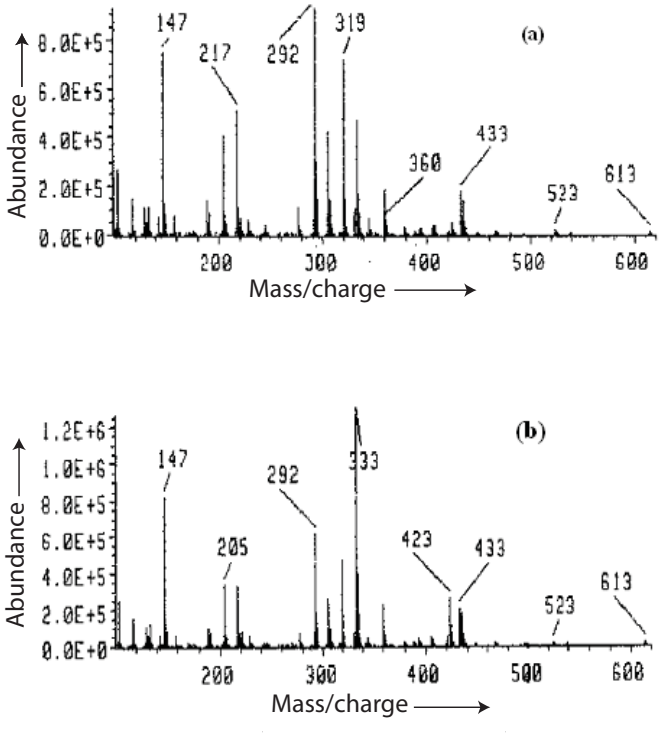

Figure 2. Mass spectrum of biologically active purified silica column material for *Ps.* str. AN5 using different aldose sugars as carbon source: (a) galactose; (b) mannose. The mass spectrum observed in (a) is identical to that of galactonic acid and in (b) to that of mannonic acid.

Colonisation of wheat roots by *Pseudomonas* bacteria

Pseudomonas str. AN5 induces erosion zones in the mucigel layer of the rhizoplane on wheat roots. A clear degradation zone is present around individual bacteria. This was consistently observed using scanning electron microscopy on the wheat root surface of plants inoculated with *Ps.* str. AN5. Transmission electron microscopy of sectioned wheat roots inoculated with *Ps.* str. AN5 showed bacteria were consistently present in epidermal cells. They were also found in the inter-cellular region of the cortex, but not as frequently. However, they were not observed in the endodermis. We have identified two Tn5 gus transposon mutants *(Ps.* str. AN5MN3 and *Ps.* str. AN5MN4) that lacked this phenotype when inoculated onto wheat roots (Table 1).

Table 1. Infection characteristic of *Ps.* str. AN5 compared to the colonisation-deficient mutants

Bacterial *Ps.* strain	Erosion zones	Wheat root invasion	
		Epidermis	Cortical
AN5	+	+	+
AN5MN3	–	–	–
AN5MN4	–	–	–

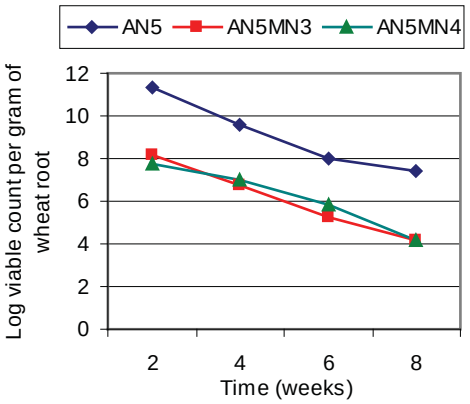

Figure 3. Root colonisation of wild-type *Ps.* str. AN5, compared to the colonisation defilement mutants *Ps.* str. AN5MN3 and *Ps.* str. AN5MN4 in pot trials in controlled environment cabinets

Both these strains had a reduced ability to colonise wheat roots (Fig. 3). *Ps.* str. AN5MN3 had similar growth characteristics to the parent *Ps.* str. AN5 *invitro.* However, *Ps.* str. AN5MN4 is significantly delayed in growth on artificial media (nutrient agar/broth; potato dextrose agar/broth), compared to parent strain *Ps.* str. AN5. *Ps.* str. AN5MN4 colonisation mutant was shown to have a single transposon insert in the DTDP-glucose 4, 6-dehydratase gene in this bacteria.

Plant gene expression as a result of *Pseudomonas* colonisation

To determine the response of the host wheat plant to *Ps.* str. AN5 inoculation, wheat seeds were sterilised and germinated on agar plates (overnight so that the root just started to emerge). The wheat seeds were then treated with *Ps.* strain AN5 grown in nutrient broth (in 3.5% methylcellulose solution to bind the bacteria to the seed). Either *Ps.* strain AN5-treated or untreated control seeds were transferred to sterile Magenta jars. The Magenta jars were placed in the same growth cabinet under controlled environmental conditions. Individual jars were demounted after 4 weeks and total RNA was isolated in duplicate from wheat roots of *Ps.* strain AN5-treated (AN5) or untreated control plants (C).

A GeneChip® experiment was performed using 5 µg each total RNA sample isolated. Four samples, named AN5(1), AN5(2), C(l) and C(2), were each hybridised to a GeneChip® Wheat Genome Array (Nayudu *et al.* 2008a). The fluorescent signals have been detected by the Affymetrix Genechip Scanner and extracted by GCOS software. The Signal and Detection of each gene are generated. The wheat genome annotation is downloaded from the Affymetrix web site and incorporated to the gene data sheet. The paired comparisons AN5(1) versus C(l), AN5(1) versus C(2), AN5(2) versus C(l), AN5(2) versus C(2), C(l) versus C(2) and AN5(1) versus AN5(2) have been done in GCOS, based on the Affymetrix algorithms. Signal Log Ratio and Change have been generated for each comparison (Nayudu *et al.* 2008a). The data mining tool Acquity 4.0 was used to perform two group *t*-tests (group 1: AN5(1) and AN5(2); group 2: C(l) and C(2)).

The gene list obtained was sorted according to Signal and Detection. About 50% of genes were absent and signals close to background across the four samples were eliminated. Then the list was sorted by the Signal Log Ratio and Change; only the changed genes with Signal Log Ratio of treatments (AN5(1) and AN5(2)) versus controls (C(l) and C(2)) larger than 1 (a two-fold change) and with no change or very little change within the treatments and controls have remained.

Most wheat genes identified from this Affymetrix analysis encode products of unknown function as analysis of the wheat genome is still in its infancy. Established Sequence Tags (EST) can be used in BLAST searches to try and identify the unknown gene. The Affymetrix analysis does identify putative gene function of well-characterised genes if they have been characterised in wheat or if their homology is similar to genes identified in other organisms (e.g. *Oryza sativa*—rice). Table 2 shows an example of a wheat gene that is up-regulated and one that is down-regulated by *Ps.* str. AN5.

The total numbers of wheat genes altered significantly (as shown by the *t*-test at two confidence levels) by to the *Ps.* strain AN5 treatment were:

Table 2. Differential pattern of RNA expression in response to *Ps.* strain AN5 inoculation. Wheat untreated is labelled 'C', while wheat treated with *Ps.* str. AN5 is labeled as 'AN5'. Gene expression is shown in standardised units.

Gene identity	Treatments			
	AN5(1)	AN5(2)	C(1)	C(1)
Putative cytochrome P450	226.4	171.0	20.6	1.7
Moderately similar to XP 462851 of *Oryza sativa* (rice), (function not known)	41.1	51.4	351.8	254.7

- 208 genes at the 90% confidence level
- 132 genes at the 95% confidence level.

To confirm these results we took four wheat genes that were altered in expression by *Ps.* str. AN5 inoculation and designed TaqMan RT-PCR probes for them. Using the RNA isolated for the gene chip experiment (Nayudu *et al.* 2008a) we were able to confirm the altered expression of wheat genes due to *Ps.* str. AN5 inoculation in three of the cases, using the TaqMan assay with RT-PCR.

Discussion

Our results strongly suggest that *Ps.* str. AN5 produces anti-fungal gluconic acid in take-all biocontrol by oxidation of the aldose sugar glucose to the corresponding aldonic acid using the enzyme GOD. The PQQ cofactor is essential for this process in donating ATP required for the enzymatic reaction involving GOD.

Pseudomonas str. AN5 is an excellent coloniser of wheat roots in different soil types and suppresses take-all disease symptoms, potentially leading to an increase in wheat yield due to biological control protection. In particular, its ability to survive well at low soil moisture content paves the way for it to be developed as a biocontrol agent against take-all for the often dry environmental conditions of dryland wheat cropping in Australia.

Pseudomonas str. AN5 has a specific process in infecting wheat roots by inducing erosion zones leading to the bacteria invading the epidermal and cortical regions. Similar erosion zones have been observed in nodulation of the non-legume *Parasponia* by *Rhizobium* (Bender *et al.* 1987). Two reduced-colonisation mutants have been identified which are impaired in this process. One of them has been characterised and shown to be in the dehydratase gene involved in LPS production in bacteria. The homologous gene in *Neisseria gonorrhoeae* has been shown to be involved in human pathogenesis, and in the case *of Rhizobium* strain NGR234 in nodulation of legumes.

Using Affymetrix genechip microarray technology we have identified wheat genes up- and down-regulated by infection of *Ps.* str. AN5 on wheat roots. We have confirmed this finding by designing TaqMan probes for a few of these wheat genes differentially expressed and using them in RT-PCR. We have also shown that bacterial genes involved in biocontrol are expressed well on wheat roots in soil in the field. Finally we have presented conclusive evidence for the first time showing that *Pseudomonas* bacterial interaction with wheat roots in the rhizosphere in biological control is a specific process in which the bacteria invade and reside inside the wheat roots. This process involves the expression of both *Pseudomonas* and wheat genes coordinately, and as such should be considered as a plant–microbe symbiosis.

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New insights into roles of arbuscular mycorrhizas in P nutrition of crops reveal the need for conceptual changes

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Abstract

Many soils are phosphorus (P) deficient. The cost of P fertiliser is increasing and the reserves of phosphate rock from which it is manufactured are diminishing rapidly. There is accordingly a major need to identify and understand traits that maximise the efficiency of P uptake by plants. Most crops, including cereals, form arbuscular mycorrhizas (AM) in the field; their symbiotic root systems are therefore the normal nutrient absorbing organs. Despite this, cereals often do not show marked positive responses to AM inoculation in the vegetative stages of pot experiments and this has led to a reduced emphasis in recent years on applied research into the roles of AM in cereal P nutrition. However, new research has provided significant insights into the ways that plant roots and AM fungi are integrated, structurally and functionally. The most important finding is that the contribution of AM fungi to plant nutrition may be large but remains hidden unless special techniques are used. We have shown large contributions of AM fungi to P uptake in both wheat and barley, even when growth and total nutrient uptake are less than in non-mycorrhizal (NM) control treatments. Furthermore, such growth depressions in AM plants cannot be explained simply by carbon (C) drain to the fungal symbionts. Future research should be directed to understanding the way in which nutrient uptake pathways through roots and AM fungi are integrated and controlled, so that new crops can be developed that capture benefits of both direct and AM uptake of P and make maximal use of nutrient resources in soils and fertilisers.

Introduction

A very large proportion of land under agriculture is P deficient and requires inputs of P fertiliser to be productive. Such fertiliser is produced from deposits of phosphate rock which represent a finite resource, with rapidly increasing price. Some calculations suggest that reserves will be exhausted in 85–190 years. These factors bring into sharp focus the need for research into mechanisms underpinning efficient P uptake by plants, including those involving their AM fungal symbionts.

AM fungi colonise about 80% of terrestrial plants, including herbs, shrubs and trees, and hence the vast majority of species in natural and agro-ecosystems. They have recently been reclassified into a separate fungal phylum, the Glomeromycota (Schüßler *et al.* 2001). Not all form storage vesicles in plant roots, so the long-established descriptor 'vesicular-arbuscular' mycorrhiza (VAM) has been replaced by 'arbuscular' mycorrhiza (AM). The new terminology recognises that many members of the

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Glomeromycota form characteristic, treelike structures called arbuscules within root cortical cells. The arbuscules are commonly believed to play major roles in nutrient transfers between the symbionts and have been used (rightly or wrongly, see below) as diagnostic of the AM symbiosis[1]. The fact that AM fungi are integral components of the large majority of normal root systems has been strongly emphasised in the past, but continues to be overlooked in both ecological and agricultural contexts. A considerable amount of research led to significant advances in our understanding of AM symbioses (Koide and Mosse 2004; Smith and Read 2008). Generalisations were made, some of which have stood the test of time, whereas others require re-evaluation. It is the purpose of this article to highlight recent advances on effects of AM symbioses in plant P nutrition and show how the new knowledge can be used to understand the roles of AM symbioses in plants, particularly in crop species, and to provide new avenues to capture any benefits.

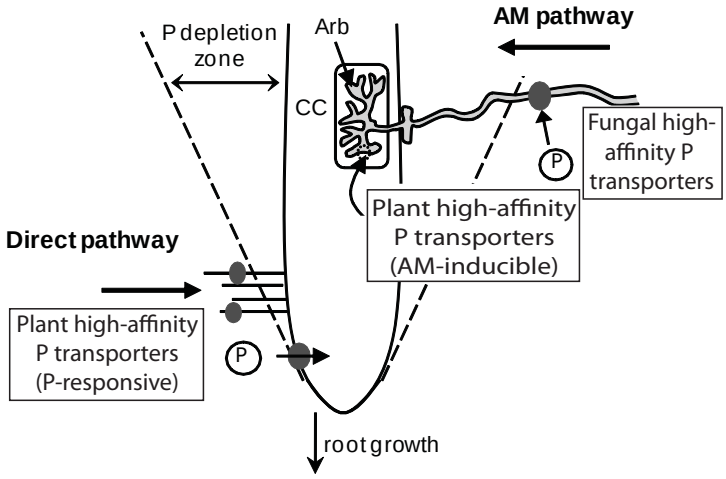

Figure 1. Diagrammatic representation of the integration of the direct and AM pathways of uptake of orthophosphate (P) in a root growing through soil. The direct pathway involves expression of plant P transporters in root hairs and epidermis and results in progressive depletion of P close to the root surface. The AM pathway involves the expression of fungal high-affinity transporters in external hyphae and plant transporters in the interface between fungus and plant. The intracellular fungus is depicted as an arbuscule (arb) inside a cortical cell (CC), but other structures (see Fig. 3) may also be important.

The conventional story

AM symbioses have been considered to be typically mutualistic, based on reciprocal exchange of soil-derived mineral nutrients and plant sugars. The main nutrients are P and zinc (Zn), both of which are highly immobile in soil. Extensive growth of the AM fungal hyphae in soil considerably extends the absorptive surfaces of the symbiotic root system and overcomes localised nutrient depletion which occurs because the rate of uptake by roots exceeds the rate of replacement from the bulk soil. Hyphae are able to access pores of considerably smaller diameter than can roots, greatly increasing the volume of the soil solution from which P can be absorbed as orthophosphate (Smith and Read 2008).

In many plants establishment of AM symbioses leads to increased P uptake and hence, when P is limiting, to increased growth. The 'conventional' view has been that P uptake by external hyphae (the AM pathway) supplements direct uptake by the plant root (the direct pathway) (Fig. 1) and that the extent of 'benefit' in terms of growth is related to the amount of AM colonisation in roots.

It was believed that AM colonisation does not affect direct uptake by the root and that activity of this and the AM pathway are strictly additive. Where colonisation is low and or total P uptake unchanged by the symbiosis, it has been assumed that the AM fungi are making a negligible contribution to nutrition. As an increased number of plant–fungus combinations was investigated a diversity of plant responses was recognised. These ranged

[1] We will follow common usage and refer to members of the Glomeromycota as AM fungi, while recognising that arbuscules are not formed in all plant-fungus combinations

from large increases in growth of AM compared to NM plants, to considerable reductions. It was again supposed (but now shown to be incorrect, see below) that where responses are neutral or negative the fungi are making no contribution to nutrition. Whereas AM fungi colonising plants showing positive benefit have been viewed as mutualistic, those colonising plants that did not respond positively have been generally believed to be parasitic (Johnson *et al.* 1997; Smith *et al.* 2009). It was also thought that the latter group use plant-derived carbon (C), proportional to the extent of fungal development.

Major cereal crops, such as wheat and barley, frequently do not show positive responses to AM colonisation in experiments investigating vegetative growth (Fig. 2a). This has led to a belief that AM colonisation is of little importance in P nutrition and productivity of these crops (e.g., Ryan and Graham 2002) and to a consequent lack of emphasis in research on the roles of the symbionts in nutrition. In our view this situation should now be reversed. Crops, including cereals, are normally AM-colonised in the field and an understanding of crop nutrition requires an understanding of how symbiotic root systems function.

It is the experimentally-induced NM condition that is abnormal, but is receiving the largest research input with respect to maximising efficiency of nutrient uptake of normally AM crops.

New insights into AM development and function in P uptake

Complexity of AM structures may have led to underestimation of incidence of AM colonisation in the field

Recent work has shown that many of the conventional views of the development of AM and their roles in nutrient uptake and plant growth must be revised. Arbuscules are not the only intracellular interfaces formed by AM fungi that are functional in nutrient transfers. In many plant–fungus combinations the major intracellular fungal development is by coiled hyphae sometimes bearing short arbuscule-like branches (Smith and Smith 1996). Both intracellular coils and arbuscules may be associated with intracellular hyphae (Dickson 2004) (see Fig. 3).

Because of this complexity and because arbuscules were long considered diagnostic for AM colonisation, the presence of AM fungi in roots has almost certainly been underestimated, particularly in field samples where AM fungi may have been recorded as absent because the only structures seen were hyphae and intracellular coils. Nevertheless, most reports indicate that cereal crops are colonised by AM fungi in the field, with values ranging from zero (questionable, considering the above) to 80% of root length (Jensen and Jakobsen 1980; Baon *et al.* 1992; Ryan *et al.* 1994; Boyetchko and Tewari 1995; Aliasgharzadeh *et al.* 2001; Li 2004). In some soils colonisation is reduced by application of P fertiliser (e.g., Baon *et al.* 1992; Ryan and Angus 2003), drought (Ryan and Ash 1996) and crop rotations involving non-AM hosts such as canola (Arihara and Karasawa 2000; Miller 2000; Ryan and Angus 2003; reviewed by Lekberg and Koide 2005), so that the significance of AM in such soils has again been questioned (Ryan and Graham 2002). However, in other soils (such as the highly P-fixing soils of the Eyre Peninsula, South Australia) colonisation is very high (up to 80% of root length) and little

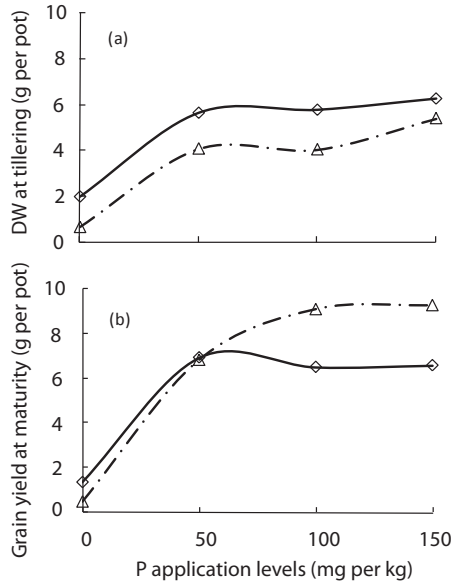

Figure 2. Growth (dry weight, DW) of wheat in a highly calcareous soil with different levels of P applied as CaHPO4, inoculated with the AM fungus *Glomus intraradices* (dotted lines and triangles) or not inoculated (solid lines and diamonds). a) dry weight (DW) at tillering; b) grain yield at harvest.

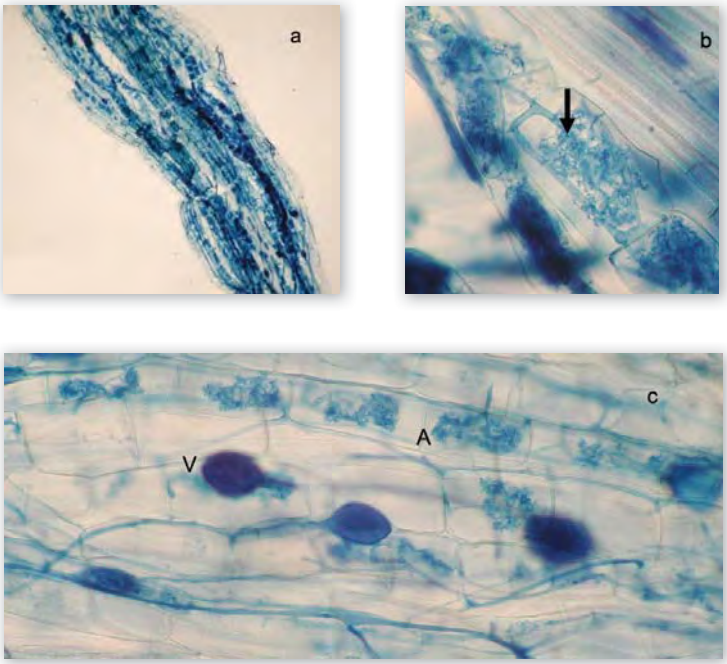

Figure 3. Mycorrhizal colonisation in wheat roots taken from the field. a) Low-magnification image showing the intense mycorrhizal colonisation (stained blue) in most cortical cells of the root. b) Detail of colonisation showing coiled intracellular hyphae bearing arbusculate branches (arrowed). c) Detail of colonisation showing intracellular arbuscules (A) and vesicles (V).

affected by addition of P (Li *et al.* 2006, 2008b; H.Y. Li, unpublished[2]) or drought (H.Y. Li, unpublished). In such situations where colonisation is high there is clearly a potential for AM to have significant effects on plant function. As shown in Figure 2b, inoculation with an AM fungus produced significant yield benefits in wheat at relatively high P application rates, even though a reduction in growth had been observed in the inoculated treatments at tillering (Fig. 2a).

Lack of direct relationship between extent of colonisation and AM effects

It has also been frequently held that there is a direct relationship between the extent (percent) of colonisation and the extent of any AM effect, such as benefit in terms of P uptake or growth. Where this relationship was not apparent the suggestion has been made that AM fungi were not functional in terms of plant nutrition and other benefits were adduced to explain the prevalence of the symbiosis (Newsham *et al.* 1995).

It has become increasingly obvious that there is no strong relationship between percent colonisation and either increased nutrient uptake at the whole-plant level, or indeed in the extent of any changes in growth (Graham and Abbott 2000; Grace *et al.* 2009a; Li *et al.* 2008a). The apparent paradox has been partially resolved by quantification of the actual contributions of the AM fungi to P uptake using radioactive P ($^{33/32}$P).

The basic principle of the technique is to grow plants in pots divided into compartments by mesh that allows hyphae of AM fungi, but not roots, to penetrate. An AM plant is grown in one compartment, where soil is explored by both roots and hyphae (root hyphal compartment—RHC). Hyphae grow through the mesh into the second (hyphal) compartment (HC). Soil in the HC is labelled with ^{32}P or ^{33}P, providing a tracer for uptake and transfer by the AM fungal hyphae. Any tracer reaching the plant must have done so via the AM pathway (Fig. 1). Results show that the AM pathway can make strong contributions to P uptake—even when the plant does not respond positively to colonisation in terms of growth or total P uptake—and that there can be considerable variations depending on the fungal symbiont (Table 1). A recent advance was made by using an HC that is very small in relation to the size of the main pot, so that its presence had little effect on the total P available to the plants (Smith *et al.* 2003). In the same investigation the contribution of the AM pathway was quantified, using specific activity of ^{33}P in soil and plants and hyphal lengths of AM fungi in both RHCs and HCs (Smith *et al.* 2003, 2004).

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Table 1. Percentage of total P taken up via the AM pathway into plants that did not show a positive growth response to colonisation in the same experiments. Quantified results are obtained by extrapolating uptake from hyphal compartments to the whole pot.

Plant	AM fungus	Fraction of total P via AM pathway (%)	Reference
Barley	*G. intraradices*	~40–55	Grace (2008); Grace *et al.* (2009b)
	G. geosporum	~ 5	
Wheat	*G. caledonium*	100	Ravnskov and Jakobsen (1995)
	G. invermaium	~0	Ravnskov and Jakobsen (1995)
	G. intraradices	50–60	Li *et al.* (2006)
Cucumber	*G. caledonium*	100	Pearson and Jakobsen (1993)
			Ravnskov and Jakobsen (1995)
	G. invermaium	0 or 20	Pearson and Jakobsen (1993)
			Ravnskov and Jakobsen (1995)
	S. calospora	~7	Pearson and Jakobsen (1993)
	G. intraradices	Not quantified	Ravnskov *et al.* (1999)
Bromus sp.	*G. etunicatum*	Not quantified	Hetrick *et al.* (1994)
Pea	3 mixed species	Not quantified	Gavito *et al.* (2002)
Tomato	*G. caledonium*	20–30	Smith *et al.* (2004)
	G. intraradices	90–100	

This work established that *Glomus intraradices* contributed up to 100% of the P reaching the plants in *Medicago truncatula*, which showed a marked increase in growth and P uptake when mycorrhizal, as well as in *Solanum lycopersicum* (tomato), which did not. Other plant–fungus combinations have shown variable, but still significant, contributions of the AM pathway (Table 1).

The quantitative approach has been extended to barley and wheat, in which the AM fungi contribute strongly to P uptake even when the AM plants show reductions in both growth and total P uptake compared with NM plants. In particular, our work has shown that the AM pathway involving *G. intraradices* contributed ~50% of P absorbed by barley (Grace 2008; Grace *et al.* 2009b) and up to ~60% by wheat (Li *et al.* 2006). In neither case was there a significant effect of P fertilisation on the contribution of the two pathways, which indicates that increasing P availability did not increase the ability of the roots themselves to absorb P via the direct pathway.

Two key conclusions must be drawn from this work:

- the AM pathway operates to provide P to plants regardless of their responsiveness in terms of growth or P uptake
- the operation of the direct and AM pathways is not additive.

If 100% of the P enters through the AM pathway, then the direct pathway must be non-functional and, furthermore, if the AM pathway is functional to any extent in a non-responsive plant (as repeatedly shown), then again the contribution of the direct pathway must be of reduced significance compared with NM plants. In other words, uptake of P (and probably other nutrients like Zn and N) via the AM pathway may be 'hidden' unless tracers are used to reveal its occurrence.

Studies of gene expression have also shed light on the operation of the AM-P uptake pathway. It is clear that the operation of the two pathways depends on expression of different genes (see Fig. 1). In the direct pathway, P is taken up via high-affinity transporters located in the root hairs and root epidermal cells. The few studies of these genes in relation to root development show that expression appears to be maximal just behind the root apices, where P in the soil solution would also be expected to be highest before rapid uptake leads to depletion of P in the rhizosphere (Daram *et al.* 1998; Gordon-Weeks *et al.* 2003). The AM pathway depends on P transporters in both fungus and plant. High-affinity P transporters have been characterised from several AM fungi. They are all expressed in external hyphae, as would be required for uptake from the soil solution (Harrison and van Buuren

1995; Maldonado-Mendoza *et al.* 2001; Benedetto *et al.* 2005; Balestrini *et al.* 2007). Uptake of P is followed by rapid translocation within the external hyphae and delivery to the symbiotic interfaces within the root cortex (arbuscules, coils and/or arbusculate coils—see Fig. 3) (Ezawa *et al.* 2002). The mechanism of loss of P from the hyphae remains a mystery, but uptake by the plant membrane in the interface involves expression of AM-specific or AM-inducible P transporters (reviewed by Bucher 2006; Javot *et al.* 2007). As AM development occurs first behind the root apex (Smith *et al.* 1986), the external hyphae and fungal structures within the root are apparently well placed to take over from uptake by the epidermal cells and root hairs near the apex as P becomes depleted and the root apex itself grows into hitherto unexploited soil. This theoretical scenario has not been demonstrated by developmental studies, but it seems clear that the potential for independent regulation of the expression of the transporter genes in the two pathways may be critical in determining the way they are integrated during development of a root system and in different plant–fungus combinations.

At this stage we have no clear picture of why P delivery by the direct pathway is frequently reduced in AM plants. Some studies indicate that expression of P transporter genes in the direct uptake pathway is reduced in AM plants (Liu *et al.* 1998; Burleigh *et al.* 2002; Harrison *et al.* 2002; Paszkowski *et al.* 2002; Glassop *et al.* 2005; Christophersen *et al.* 2009). However, others show no such effect, even with the same plant–fungus combinations (Karandashov *et al.* 2004; Nagy *et al.* 2005; Poulsen *et al.* 2005; Grace 2008; Grace *et al.* 2009b). As highlighted frequently in the past, it may be that soil conditions and extension of the P uptake surfaces (roots or hyphae) play a much more significant role in controlling P uptake than the kinetic characteristics of the transporters (Clarkson 1985; Silberbush and Barber 1983; Raghothama 2000; Tinker and Nye 2000). This is an important area for future research.

Growth depressions

Although negative effects of AM colonisation on plant P uptake and growth have been recognised for a very long time (Smith 1980), they have received little attention and may even have been played down in published research because they were thought to be artefacts of experimental conditions and appeared to detract from the positive image of the symbiosis and potential for management to optimise the benefits. This blinkered vision meant that a whole range of interactions between AM fungi and plants was excluded from study, with consequent loss of key information that might lead to understanding of their causes (see Johnson *et al.* 1997; Jones and Smith 2004; Janos 2007; Smith *et al.* 2009). This is not the place to discuss the complex physiological issues involved. It is important to point out, however, that the most common explanation for depressions has been that the plants are C-limited and that the fungi exert a C drain on the plants that is not offset by increased nutrient uptake (Smith 1980; Bethlenfalvay *et al.* 1982). Hence, extensive and rapid colonisation was thought to be a key contributor to AM depressions in growth, especially in soils where nutrients were readily available and hence AM symbiosis 'not required for uptake'. We now know that the fungi are involved in P uptake and we also point out that growth depressions are often transitory (compare Figs 2a and 2b) and occur both in plants that are extensively colonised and in those that are not (see Graham and Abbott 2000; Grace 2008; Li *et al.* 2008a; Grace *et al.* 2009b; Smith *et al.* 2009). As with P uptake, the conventional explanation is not adequate to explain the range of situations under which growth depressions occur. The issue is important because a number of staple crops, including wheat and barley, are known to show depressions in experimental conditions. What is required is an understanding of how activities of the symbionts are controlled and integrated in the field where AM symbioses are normal.

Conceptual changes are required

The recent work reviewed here indicates that conceptual changes in our understanding of the operation of AM symbioses in field situations are necessary:

- First and most important, the vast majority of plants (including crops) are AM-colonised in field situations. If we are to understand crop nutrition and growth in the field we must understand how the activities of AM fungi and plants are integrated over a very diverse range of responses.
- A range of different intracellular structures are formed in root cortical cells by AM

fungi. Identification of a root as 'arbuscular mycorrhizal' requires that arbuscules, coils and arbusculate coils are all recognised as functionally important.

- Effects of AM symbiosis on both nutrient uptake and plant growth (whether positive or negative) are not directly related to the extent of colonisation of the roots. Thus the 'importance' of AM and potential role in nutrient uptake cannot be gauged from the percent colonisation.
- The two pathways of nutrient uptake in an AM root (direct and AM) do not act additively, but are independently regulated. The activity of the direct pathway is frequently reduced in AM plants, regardless of the overall outcome of the symbiosis. It is therefore not possible to determine the contribution of AM symbiosis (i.e. the AM pathway) by comparing total P uptake in AM and NM plants.
- Because the AM nutrient uptake pathway operates in negatively responsive plants it is not appropriate to regard the fungi in these associations as fully parasitic. The associations are mutualistic at the cellular level, with reciprocal transfers of P and C between the symbionts. Furthermore, C-drain to the fungal symbiont is not always an adequate explanation for growth depressions in AM plants.

Implications for future work on crop plants

It is undeniable that the vast majority of major crops, including cereals, are capable of forming mycorrhizas and will do so in cropping situations in the field. Nutrient uptake in the field will therefore involve symbiotic AM root systems. As we have emphasised, the new work indicates a major influence of AM colonisation both on the pathways of nutrient uptake, including genes expressed, and on the quantitative contributions of the two pathways to P uptake. The AM pathway is involved in uptake of P whether or not the plants are responsive to AM fungi in terms of growth. Optimising the use of nutrients accumulated in soil or applied as fertiliser depends on understanding the symbiotic uptake processes and their control. If the AM and direct pathways are complementary and the AM pathway makes a major contribution, then P uptake via the direct pathway may be very low or even inoperative. Under these circumstances there is little point in research to maximise activity of the direct pathway without taking the effects of the symbiosis into account. A future research challenge will be to maximise uptake by both pathways in a way that will enhance the overall efficiency of the root systems. The aim should be to maximise the use of pools of P stored in soil, thus minimising the need for applied fertiliser and offsetting the rising costs associated with its application.

Acknowledgements

Emily Grace is grateful for a Commonwealth Hill Postgraduate Research Scholarship. We thank the South Australian Grains Industry Trust, the Australian Research Council and the Australian Centre for Plant Functional Genomics for financial support.

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Two parallel rhizosphere programs

R.J. (Jim) Cook[1,2]

Abstract

Complementary research programs at Adelaide and Pullman, led respectively by Albert Rovira and the author, have cooperated for 40 years to address significant problems of disease and yield in wheat crops.

We identified the microorganisms associated with take-all decline in wheat, and observed the complexity of the interaction between the root-damaging organisms and cultivation and cropping history. We showed that soil sterilisation could enable crops to more fully exploit the nutrients available in the soil. We have been able to identify at least some factors important to the successful use of low-tillage systems for wheat cropping.

Albert Rovira has made a seminal contribution to our understanding of the rhizosphere in cereal cropping.

Introduction

Congratulations on the symposium — it is a wonderful tribute to Albert Rovira.

I am very sorry I can't be at the event, although you have a great representative from the Pacific Northwest in Dick Smiley. I thought that doing a DVD or video would be the next best thing, and what better place than in my favourite habitat—right here in the wheat fields.

I remember first meeting Albert Rovira at the Berkley Symposium in 1963. Albert was fresh into his new job at CSIRO as a rhizosphere microbiologist after coming from a post-doctorate in Sweden. During his presentation Albert showed pictures of bacteria on the root surface—little islands of bacteria jockeying for space. We were all amazed to see this first presentation of what bacteria looked like on the surface, and since then Albert has just gone on to show so much more in this area.

It was quite surprising that within one year I would be in Australia working with Noel Flentje at the Waite Agricultural Research Institute just across the road from Albert's lab at CSIRO Division of Soils. I especially remember going along to see one of his field trials with wheat inoculated with bacteria, trying to confirm some of the Russian work on growth promotion through N fixation provided by a bacterium applied to the seeds. At that time, all of his work was in the realm of basic or fundamental research. That's one of the reasons that I enjoyed looking at the sort of things he was doing. I think it started with Dick Smiley doing his PhD with me, and then getting down to Australia—it may have been in 1972–1973—and doing a post-doctoral

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with you, Albert, that you began to change the way you looked at soil microbiology. That was the period when you began to critically seek answers to the question of what limits crop productivity—whether fertility or soil biology—as opposed to but probably building on your experiences in the more fundamental chemical and biological processes of the rhizosphere. It was a very major change. It was a real tribute that, after working 15 years in one area and 15 years in another area, you became a Fellow of the American Phytopathological Society for your work on rhizosphere pathology. I know you are very proud of that and I'm very proud of that too.

I will talk about three different projects that I think have tied our programs together. These are:

- the first evidence of the role of fluorescent pseudomonads in take-all decline
- research on soil fumigation that led to the diagnosis of the ubiquitous presence of *Pythium* root rot here in eastern Washington, adjacent Idaho and Oregon
- work on the problems encountered with no-till farming and discovery of the role of and means to control *Rhizoctonia* root-rot.

These are all major topics, each one of which would deserve a full hour of conversation.

The role of fluorescent pseudomonads in take-all decline

My interest in take-all decline was really sparked when I met Peter Shipton in 1968 at the First International Congress of Plant Pathology. Peter had just done his PhD on take-all decline following the work of Gerlagh in the Netherlands, and had a soil assay system to identify fields in take-all decline. It was only a year or two later that Peter joined my program at Pullman and we took up the whole question of what would happen in response to continuous wheat cropping with respect to take-all. Dick Smiley will remember very well the classic experiment that we did because he helped us to set it up, using soils from six different fields in eastern Washington. One of these fields was later nicknamed the Quincy Field (QF) where the farmer had grown wheat for 12 consecutive years and where take-all was not a problem, although we never knew whether it ever was a problem. The other five fields had a history either of no wheat or wheat in a crop rotation. We moved several hundred kilos of each of these soils to western Washington, to the WSU Research Center at Puyallup. Dick also did one of his experiments on the influence of form of nitrogen on rhizosphere pH right alongside a site where we mixed in those six different soils in four replicate blocks. In the first year, we introduced the pathogen as colonised oat grains at the time of planting and the wheat was devastated from one corner of the experimental site to the other by take-all. But the second year, when we dug up the plants, we were amazed, as you will see (Fig. 1), the roots were white exactly and exclusively from the four plots that received soil from that field of 12 years of continuous wheat, QF, but where we did not add any soil, or where we added the Quincy Virgin (QV) soil from just across the fence line, or any of the other four soils, these soils contributed nothing to take-all suppression. I'll never forget those white roots in those four plastic bags with plants that came from those four plots — the white roots just like you see in the picture.

Figure 2 gives a view the profile of the wheat in the plots after heading. The plots were 1.5 m wide and 3 m long. We mowed out the front and held up some butchers' paper behind the standing wheat to get a profile view of the wheat as it was starting into heading.

Figure 1. Wheat roots black with take-all (left and centre) and healthy white (right) from a field plot where, two years earlier, soil from a field cropped to wheat for 12 consecutive years had been mixed to 15 cm deep at about 0.5% by weight. The checks had no foreign soil added (left) or soil was added from a site next to the long-term wheat field but never planted to wheat (centre).

Figure 2. Replicate 3 (centre), typical of all four replicates. The tall plants are in a plot where soil from a field cropped to wheat for 12 consecutive years had been mixed in two years earlier; in comparison the stunted wheat, severely affected by take-all, is in plots where other soils or no soil had been mixed in previously (see Fig. 1).

The wheat in plots amended with the QF soil was clearly taller, and it was because of those white roots. Take-all suppression took two years to manifest itself in these plots, but in the third year (Lex Parker was there to see it), it was non-stop take-all decline from one end of that experiment to the other. That was really the start of my interest in studying microbial processes through the transfer of soil. A year later I was in Australia in Albert's lab ready to go to work on that very question.

Research on soil fumigation

I recalled Rex Krause from his days at Pullman, so we went out to Roseworthy Agricultural College and Rex helped me get some soil that was beautiful soil to work with—coarse and well drained—to use as a fumigated rooting medium. We will never forget those two 3 lb Maxwell House coffee cans that I shipped in advance of my arrival, one full of QF soil and the other full of QV soil. Albert had them in the cold room when I arrived. I got the soil from Roseworthy and mixed it up with the QF soil and the QV soil. Meanwhile, Albert and I, with others, made a trip to Horsham where the Research Centre had grown wheat repeatedly in the same fields for 50 years. We obtained some of that (H) soil. This resulted in a pot trial—Figure 3.

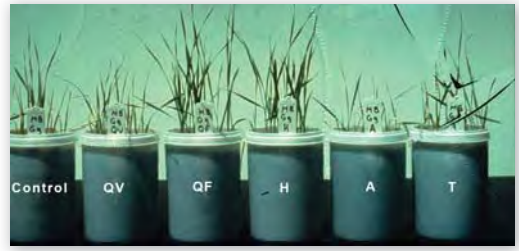

Figure 3. Pot experiment done in Albert's lab in 1973, where soil from Roseworthy College and fumigated with methyl bromide (Mb) was used as the common rooting medium amended with the take-all pathogen (Gg) and then also amended at 1% by weight with soil never cropped to wheat (QV), cropped continuously to wheat (QF and H), or cropped to wheat as a component in a crop rotation (A and T). The control received no soil amendment.

I was invited to give a seminar at CSIRO just as I was completing this trial. I had the pots on a bench beside the podium with brown paper in front of them, and I gave my seminar about the transfer of soil at Puyallup and then I talked about the pot test. At the right time, I pulled that brown paper up. You could clearly see the suppression of take-all with the two soils that came from the fields cropped continuously to wheat, but not with soil from fields where wheat had not been grown regularly or at all.

I looked at Albert in front of the audience, and said 'I've come to work with the world's best rhizosphere microbiologist to find out what was in that tablespoonful of soil'. That got us going. Even today with take-all decline, we have never been able to actually introduce the agent by seed inoculation. With the story about the antibiotic and so forth, 'take-all decline' is now the centre-piece for our intensive

Figure 4. The plot fumigated with Telone C17 yielded 8 tonnes/ha (upper plots) vs. 6 tonnes/ha in unfumigated soil (foreground).

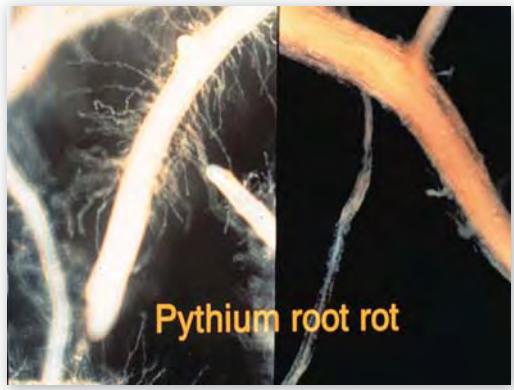

Figure 5. Healthy white wheat roots from fumigated soils (left); *Pythium*-affected roots stripped of fine rootlets and root hairs from unfumigated soil (right)

direct-seed cropping systems here in the Pacific Northwest. Then superimposed on that are all the other things we have to do to control the other root diseases, so we can grow cereals continuously with good fertility.

Dick Smiley, working with Noel Sutherland at CSIRO, started to experiment with soil fumigation when he joined Albert's lab. My understanding is that it was from these early experiments that Albert got the idea for his program to look at root diseases including the nematodes. I was amazed at Albert's field trials done collaboratively with Ted Ridge; when I came back in the seventies I started my own fumigation trials just out of Pullman with the help of Bill Haglund. In Figure 4, you can see one of the wheat fields where we showed a two-metric-tonne yield increase from just fumigating the soil. This response was in a three-year rotation.

We got 8 tonnes/ha where we fumigated compared to 6 tonnes where the soil was left natural. Six tonnes is very respectable but 8 tonnes was the potential yield with the same fertiliser and water. There was some nitrogen left unused in the non-fumigated plots, as Albert's own work had also shown.

There was something else going on, and that led to our discovery of *Pythium*. You can see a comparison of the roots in Figure 5—white roots where *Pythium* was eliminated with Telone C17 at 240 L/ha compared with brown roots in the controls. These results are from of one of our fumigation trials set up the next year and across the road from the field shown in Figure 4.

Figure 6. Conventional no-till wheat (left) and wheat undersown with granular metalaxyl (four rows on the right)

We used the fumigant Telone C-17, which led to beautifully white roots, while the controls produced those brown roots. We discovered that *Pythium* strips off the fine rootlets and root hairs, greatly reducing the ability of the crop to get its nutrients—especially relatively immobile nutrients such as phosphorus. This shows why there was nitrogen fertiliser left unused in the soil.

The real break came with the availability of metalaxyl which was then being developed by Novartis, now known as Syngenta, specifically for control of *Pythium* and its close relatives. When I laid down metalaxyl in a granular layer under the seed in four rows, the crop looked as though we had added extra fertiliser to those rows (Fig. 6).

Figure 7. Young barley plant showing classical symptoms of *Rhizoctonia* root rot

Figure 8. Plots with alternating strips of conventional tillage (healthy wheat) and no till (wheat stunted with missing plants), with an area fumigated with methyl bromide across all strips (foreground)

This and similar studies led us to conclude that that *Pythium* is present everywhere and all plants are damaged more-or-less to the same degree. We had come to think of our 6 tonne/ha wheat as normal healthy wheat when in fact it should have been 8 tonnes. That was a major break-through for us; I wouldn't have done that if I hadn't become excited when I saw Albert's own fumigation trials in the 1970s.

Problems with no-till farming

As was known in both Australia and the Pacific North-West, no-till farming was starting to take off where one could farm with a planter, a sprayer and a combine, leaving all the other equipment out in 'the back 40', where it would 'rust in peace'. Of course there were some problems as well.

In 1983 Dick Smiley was on sabbatical in Melbourne, David Weller from my lab was working with Albert and I came down for the Fourth International Congress of Plant Pathology. We were introduced to the work that Albert was doing on soil disturbance for *Rhizoctonia* control.

Back home, we tried some of these experiments ourselves. We found young wheat and barley plants with exactly the same symptoms of *Rhizoctonia* root rot (Fig. 7) that wheat and barley plants showed in no-till fields in Australia.

The *Rhizoctonia* in Washington is the same anastomosis group (AG 8) as the *Rhizoctonia* in South Australia. Moreover, the SARDI DNA tests for *Rhizoctonia* in Australia detected our *Rhizoctonia* in soils here in the Pacific Northwest. This means we have not only the same pathogen, but also the same biotypes or sub-biotypes of *Rhizoctonia solani* AG 8.

I then did my own experiments comparing the effects of tillage and no-till on *Rhizoctonia* root rot of wheat. In Figure 8 you can see the strips of alternating no till, where the wheat is stunted or plants are missing, and till where the wheat looks good. The fumigated plots (in the foreground) showed that the poor growth of wheat in no-till was due to disease—not a physical effect of the undisturbed soil. This study was done at the WSU experimental station near Lind, WA, in one of my field sites that had been cropped continuously to wheat for about 20 years, and showed what *Rhizoctonia* could do without soil disturbance.

That study prompted us to pick up on Albert's sowing points used as openers for soil disturbance within the seed row; trying to 'have our cake and eat it too'—disturbing the soil only in the seed rows (but otherwise calling it no-till) and planting the seed and fertiliser all in a single pass.

The story would not be complete without talking about the 'green bridge'—what was known in Australia as 'spray-seed' and I think became 'spray wait'. Dick Smiley started experiments at Pendleton on that shortly after he got back from Australia. I, with the weed scientist Alex Ogg, did similar work. Results of an experiment that Alex and I did at the WSU experimental station at Lind are shown in

Figure 9. Barley seeded no-till in the spring where volunteer cereals had been sprayed out the previous fall (background) and only three days before planting in the spring (foreground)

Figure 9. In the background of the photo we had sprayed out volunteer *Hordeum* in the fall, whereas in the foreground it was sprayed in the spring just three days before planting.

What an incredible difference just getting rid of that volunteer crop makes! Figure 10 is an earlier photograph, of wheat planted directly into wheat stubble of the previous year's crop, in which you could see every combine row where the straw had lain after combining. The explanation at that time was that the straw—or more likely the concentration of chaff left behind the combine—was somehow toxic to wheat. Ron Kimber was working on it in Adelaide and Jim Lynch and Lloyd Elliott at Pullman. But all that time the stunting and slow maturation of wheat in the combine rows was due to root diseases favoured by the green bridge, and not to straw and chaff. It was the volunteer crop, most concentrated in the combine row, that was giving us that effect.

Soil disturbance in the seed row within an otherwise no-till system is working—we are still getting effects from that, although there is still some controversy about low-disturbance drills preferred by some vs high-disturbance drills favoured by others. I favour high-disturbance drills, at least when first getting into no-till cropping systems.

Figure 10. Wheat seeded no-till into stubble of the previous crop, showing the so-called 'combine row effect' owing to the concentration of straw left by the harvester and where plants are stunted and slow to mature (green strips). Healthier wheat is maturing more rapidly between the strips where straw and chaff were left most concentrated.

That brings the story to 2008. For the most part, you can now drive in the fall through eastern Washington and you can see the difference between the no-till fields of those who allow the stubble to green up and of those where the stubble is brown, having been sprayed in the fall—at least when there was something to spray in the fall or early in the spring.

Conclusion

My presentation has covered some of the history of our two complementary programs. We have been working together for 40 years, and our programs have run in parallel, almost in lock step, charging each other's batteries and staying the course. We have never looked back to the old-fashioned way of working up the ground and kicking up the dust. Let's get on with no-till. Let's resolve the problems so this system really works.

I say to you, Albert, 'Good luck and all the best', remembering the trip we did during your time in Pullman in 1987, from sun-up to sundown through the countryside, ending up in Idaho at sundown. These were the things we enjoyed and we remember.

Root diseases, plant health and farming systems

Richard W. Smiley[1]

Abstract

Root diseases continue to hinder the production of small-grain cereals and especially the transition from cultivated to direct-drill dryland farming systems. A deeper understanding of ecosystems and development of improved support systems and farming practices remain critically important as farmers strive to elevate disease management capabilities to a higher plateau. The focus of this paper is on root disease management practices and methods that were strongly influenced by the many contributions of Dr Albert Rovira. His career has been replete with productive collaboration among scientists, farmers and providers of farm advisory and supply services throughout Australia and internationally, as most recently delivered through The ATSE Crawford Fund. Examples in this paper are drawn mostly from rainfed wheat-based cropping systems in a low-rainfall region of the Pacific Northwest USA where root disease management was also improved through the influences of Albert Rovira and his colleagues, particularly those at Glen Osmond. The emphasis of this paper is on take-all, *Rhizoctonia* root rot (bare patch) and cereal cyst nematode but includes *Pythium* root rot, *Fusarium* crown rot and root-lesion nematode. Recent progress has included a deeper understanding of pathogen population dynamics and disease resistance, as well as development of DNA markers, pathogen detection and quantification procedures.

Introduction

Cropping systems are an integration of management decisions and practices involving a complex matrix of farm-level environmental, financial and social parameters (Howden *et al.* 1998; Pannell *et al.* 2006). Cropping system diversity occurs even among neighboring farms. Small differences in practices can directly influence interactions among micro-environments, crops and pathogens that determine plant vigor and the occurrence and severity of disease. The challenge is to develop practical and profitable disease management practices that fit within the financial and social parameters that influence decisions made by farmers. Dr Albert Rovira's contributions to agriculture and to science have clearly demonstrated that he is a scientist who was capable and willing to commit a lifelong passion to achieve this mission.

This paper addresses direct and indirect influences of Australian scientists and farmers on disease management programs in the inland Pacific Northwest USA (PNW: Idaho, eastern Oregon and eastern Washington), where wheat is the primary economic enterprise and is produced in farming systems similar to the diversity of those in the wheat belts of southern and western Australia (Freebairn *et al.* 2006; Schillinger *et al.* 2006).

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Cropping systems and plant health

Climate, enterprise profitability and resources such as soil, water, equipment and financial credit are major determinants of the complexity of enterprises on each farm. While rotation of three or more crop species in long rotations is a basic tenet for producing healthy crops globally, profitability and resources often restrict the use of effective rotations to areas having adequate precipitation or sufficient water for supplemental irrigation. In dryland farming regions receiving low annual precipitation, combinations of fragile soil, profitability of limited crop species, range in temperature, and or poor temporal distribution of precipitation often limit crop diversity. In low-rainfall regions of the PNW (150–300 mm y^{-1}) the two-year monoculture of wheat and long fallow continues to be most profitable and to have least variability in season-to-season grain yield and economic risk, albeit at the expense of declining soil quality. The area of chemical fallow has increased but remains less than cultivated fallow because direct-drill systems are often less profitable than cultivated systems, even though they reduce requirements for labor, fuel, machinery and tractor horsepower, and retain greater amounts of water in the soil profile (Machado *et al.* 2007; Schillinger *et al.* 2007; Bewick *et al.* 2008).

More intensive systems have been introduced to PNW regions with annual precipitation of 300–450 mm but most farming systems continue as functional cereal monoculture because other crops are significantly less profitable and often also reduce the yield of the following wheat crop, either by extracting too much stored water or by increasing the population of pathogens or parasites with broad host ranges, such as root-lesion nematodes. True rotations using multiple crop species occur only in areas of highest rainfall (450–600 mm y^{-1}), where profitable crops include barley, food legumes and oilseed crops. Summer crops are generally not profitable for broadacre PNW farms because night temperatures are too low and in deep soils the summer crops deplete stored water otherwise available for a subsequent wheat crop. Long rotations generally improve yields of all crops in higher-rainfall districts, but cereal crops are generally most profitable and dominate the rotational sequence. In all rainfall districts, practices for managing root diseases must be compatible with and complement practices for achieving maximum agronomic yield and for managing weeds, insect pests and foliar diseases (Ogg *et al.* 1999).

The frequent occurrence of root disease complexes involving multiple diseases and pathogen species underscores the need for crop management practices that focus on holistic root health rather than a disease that is dominant at any particular time. This has been a lifelong objective of programs led and influenced by scientists such as Albert Rovira in South Australia and R. James (Jim) Cook in the PNW (Cook and Veseth 1991). Discussions of individual diseases in this paper are limited to PNW research that was strongly influenced by studies of Albert and his colleagues at CSIRO, SARDI and the University of Adelaide. The focus is on take-all, *Rhizoctonia* root rot and cereal cyst nematode but includes research on *Pythium* root rot, *Fusarium* crown rot and root-lesion nematode. Reviews by Smiley *et al.* (2009) and Smiley and Nicol (2009) and additional PNW-oriented references in the text provide entry points for examining these topics in greater detail.

Root diseases

Take-all

Most irrigated wheat in the PNW does not experience economically important damage from take-all due to the low frequency of wheat in these high-value cropping systems. However, high wheat prices cause some farmers to plant wheat frequently or repeatedly, occasionally leading to destruction of the crop by take-all. But, as in Australia, take-all is also widespread and causes chronic damage in even the lowest-rainfall regions of the PNW (Paulitz *et al.* 2002; Cook 2003). 'Dryland take-all', as named by C.A. 'Lex' Parker in Western Australia, occurs where environmental conditions favor infection of roots that contact soilborne inoculum (primary infection cycle) but are unfavourable for subsequent root-to-root and up-the-stem spread (secondary infection cycle) that occurs in higher-moisture environments.

Take-all in the PNW has either been unaffected by tillage intensity or more prevalent in direct-drill than cultivated systems (Schroeder and Paulitz 2006). Damage to roots is reduced when starter fertiliser is banded directly below the seed, regardless of the application procedure or timing for the remainder of the fertiliser requirement. Take-all decline with wheat monoculture occurs in PNW regions that receive supplemental irrigation or more than 550 mm y^{-1} of precipitation (Weller *et*

al. 2007), but not in soils that have a combination of low microbial activity due to low organic matter content (<1.5%), very dry surface horizons during warm weather, and very low temperatures when the soil is moist. Predictive models developed and used in Australia (by David Roget, Herdina, Kathy Ophel-Keller and Alan McKay at CSIRO and SARDI) and in Europe (by Alexandra Schoeny and Philippe Lucas at INRA, Le Rheu, France) have not yet been adapted to the PNW.

The most intensive and sustained take-all research in the PNW has focused on antagonists of *Gaeumannomyces graminis* var. *tritici* (Cook 2003; Weller *et al.* 2007). The role of antagonistic fluorescent *Pseudomonas* species has been revealed in great depth over the past 40 years, after first being detected during the 1970s by scientists in Albert Rovira's CSIRO laboratory. That work, to which I once contributed (Smiley and Cook 1973; Smiley 1978a,b, 1979), set the stage for exhaustive global attempts to manage these antagonists as bio-control agents. In addition to the CSIRO lab formerly directed by Albert, a focal point for this management approach has been the USDA Biological Control and Root Disease Research Lab at Pullman, Washington. That lab was developed and formerly directed by Jim Cook. More than 22 genotypes of 2,4-diacetylphloroglucinol (2,4-DAPG)-producing *Pseudomonas fluorescens* are now recognised as having an important impact on root health and particularly in protecting roots against infection by the pathogen. Competitive interactions between the pathogen and specific 2,4-DAPG genotypes in the rhizosphere are well defined (Weller *et al.* 2007) and emphasis has been placed on managing native populations of these antagonistic microbes in soil (Cook 2006, 2007), using tools such as managing rotations, soil reaction and the dominant molecular form of nitrogen absorbed by roots.

Rhizoctonia root rot

In the PNW the acute stage (bare patch) of *Rhizoctonia* root rot is observed less commonly than the chronic stage in which roots are severed on plants that are individually stunted within the drill row, without evidence of a clustering of stunted plants characteristic of the bare-patch symptom (Paulitz *et al.* 2002). The most important pathogens associated with *Rhizoctonia* diseases of rainfed cereals in the PNW include *R. solani* AG 8 and AG 2-1, and *R. oryzae* (Smiley *et al.* 2009). The dominant pathogen is AG 8 in low-rainfall regions where the bare patch symptom is commonly expressed, and AG 2-1 in higher-precipitation regions where chronic root debilitation occurs without expression of the bare-patch symptom. *Rhizoctonia oryzae* causes a cortical rot similar to AG 8 but seldom severs the root axis to cause the 'spear-tip' symptom commonly associated with AG 8. Damping-off is a symptom caused by *R. oryzae* but generally not by *R. solani* AG 8. Barley is much more susceptible than wheat, particularly in direct-drill cropping systems.

An observation first made by Albert Rovira and David Roget, among others, was transferred to the PNW and after further study was developed into what is now the most influential and cost-effective strategy for managing *Rhizoctonia* root rot. In the PNW the principle is known as 'managing the green bridge'. *Rhizoctonia* root rot is especially pronounced when fields are planted shortly after a herbicide is applied to kill volunteer cereals and or weeds infected by *Rhizoctonia*. This commonly occurs when sowing spring cereals during a narrow window between rain events in early spring, where roots of newly emerging seedlings are particularly vulnerable to infection by the enhanced level of *Rhizoctonia* inoculum that develops when natural defense mechanisms become impaired as weeds and volunteers die from the pre-plant herbicide application. The dying plants serve as highly effective bridging hosts to greatly amplify the amount of damage to emerging seedlings unless the time between herbicide application and planting is extended to an interval exceeding two weeks. Fields maintained without living hosts through the entire winter can produce yields of spring cereals twice those in fields where the volunteer and or weed hosts were sprayed only a few days before sowing. The same principle occurs in winter wheat–fallow rotations planted to 'Clearfield' wheat cultivars that are tolerant to the herbicide imazomox. These cultivars provide important opportunities to eliminate winter-annual weed species by over-spraying the winter wheat crop with imazomox. There have been notable instances in which *Rhizoctonia* damage became greater on winter wheat seedlings following application of imazomox to eliminate downy brome or goatgrass, but it remains unclear as to whether this was a direct response to a weakened natural defense in wheat roots or to a greater level of inoculum as the *Rhizoctonia* transferred from dying

weed grasses to adjacent wheat seedlings. A similar effect is likely if or when glyphosate-resistant wheat or barley cultivars become registered for release into commercial agriculture.

Effects of crop rotation have been variable because the most virulent of these *Rhizoctonia* species have broad host ranges including wheat, barley, field pea, canola, mustard and others (Paulitz *et al.* 2002; Schillinger *et al.* 2007; Smiley *et al.* 2009). As first reported in Australia, seedling health in *Rhizoctonia*-infested soil in the PNW is improved by tilling soil shortly before planting, using drill openers that vigorously stir soil in the seed row, and banding starter fertiliser below the seed. A microbial-based disease decline phenomenon was demonstrated by David Roget in the Avon Experiment in South Australia and by our group in the PNW. An initiative toward controlling *Rhizoctonia* root rot through biological means became short-lived in response to the untimely death of Dr Andrew Simon as he expanded his studies of antagonistic *Trichoderma* species at Pullman, Washington.

The most promising strategy for managing *Rhizoctonia* root rot in the PNW currently resides in further development of a wheat selection that exhibits resistance to both *R. solani* AG-8 and *R. oryzae*. This non-GMO resistant wheat was selected by Washington State University and USDA-Agricultural Research Service scientists. Doubled haploid populations were generated to develop a molecular marker for tracking the single co-dominant resistance gene in breeding programs. This prospective breakthrough was coupled with a public-domain real-time PCR procedure (Okubara *et al.* 2008) now being used to improve understandings of pathogen diversity and geographic distribution, and to promote an accelerated rate of adoption for molecular technologies in commercial testing services, following services already available through the C-Qentec/SARDI Root Disease Testing Service in Adelaide.

Cereal cyst nematode

Farmers and scientists in South Australia and Victoria have a long history with the cereal cyst nematode, *Heterodera avenae*. An extensive knowledge base and the resulting deployment of resistance genes and crop rotations have greatly diminished the amount of damage once caused by this plant-parasitic nematode. *H. avenae* was discovered in the PNW only recently. Important crop losses occur in only a few areas because long fallow is a dominant production practice and highly infested soils occur mostly in areas where crop rotations are possible. Also, the more fertile and deeper soils of the region promote more robust plants than most soils used for broadacre crops in Australia. Greatest damage occurs when farmers in highly infested areas attempt to achieve maximum farm profitability by maintaining a high frequency of wheat in the cropping system.

The SIRONEM bioassay developed by Albert Rovira and Andrew Simon became superseded by DNA-based assays such as those used by the C-Qentec/SARDI Root Disease Testing Service. Comparable diagnostic procedures are being developed in the PNW and these efforts quickly revealed the first-known occurrence of *H. filipjevi* in North America (Smiley *et al.* 2008). The impact of this additional complexity on wheat production practices has not yet been investigated.

Heterodera avenae populations tested thus far in Oregon are unable to effectively multiply in wheat lines carrying the *Cre1* resistance gene and in barley lines carrying the *Rha2* and *Rha3* resistance genes. The *Cre1* gene was recently transferred from the Australian cultivar Ouyen into PNW-adapted cultivars. A non-proprietary molecular marker is being developed to improve the efficiency of these breeding objectives.

The contributions of Albert Rovira and his many colleagues invariably fostered expansion of research efforts to include diseases that were either less known or of lower priority early in Albert's career. These diseases are now considered to be important constraints to wheat and barley health in each country and are included in this recognition of Albert's accomplishments and global influence.

Root-lesion nematode

Pratylenchus neglectus and *P. thornei* have been detected in 90% of PNW fields and potentially damaging populations occur in 60% of the fields. Procedures and Australian wheat cultivars described by Sharyn Taylor and Vivien Vanstone at SARDI and the University of Adelaide were used to demonstrate that *P. neglectus* and *P. thornei* each reduce wheat yields in the PNW without caus-

ing specific or diagnostic symptoms (Smiley and Machado 2009; Smiley and Nicol 2009; also see the author's website). Winter and spring wheat yields are frequently correlated inversely with numbers of root-lesion nematodes in soil. High populations occur at depths of up to 90 cm in the soil profile. Barley and safflower suppress the population density and canola, mustard, chickpea and wheat produce high *Pratylenchus* populations, as was previously shown throughout Australia. Procedures of John Thompson (QDPI, Toowoomba) were used to define tolerance and resistance characteristics for wheat and barley in the PNW (Sheedy *et al.* and Thompson *et al.* in *Plant Disease Management Reports* volumes 1–3, at http://www.plantmanagementnetwork.org/pub/trial/pdmr/). A wide range of tolerance levels were detected among spring wheat and barley cultivars, and tolerance to one *Pratylenchus* species was not always indicative of tolerance to both species (Smiley 2009). This species-specific tolerance for individual wheat cultivars is problematic because most commercial nematode diagnostic laboratories in the PNW will not differentiate these *Pratylenchus* species, undermining the utility of published cultivar recommendations specific to the *Pratylenchus* species occurring in individual fields. We therefore developed a robust PCR procedure to distinguish these species (Yan *et al.* 2008) and are currently transforming the procedure into a non-proprietary real-time PCR format. Also, since no current wheat cultivars adapted to the PNW exhibit a significant level of genetic resistance, crosses were made to improve the level of resistance in locally-adapted cultivars. Gene donors included Virest for *P. neglectus*, GS50A and OS55 for *P. thornei*, and AUS28451R, CPI133872 and Persia 20 for both species. Mapping populations are being used to develop molecular markers for the sources of dual-species resistance. All of this work was based upon pioneering investigations and resources developed in Australia and at CIMMYT.

Pythium diseases

Pythium damping-off and root rot are caused by a complex of 13 species in the PNW (Paulitz *et al.* 2002; Schroeder and Paulitz 2006; Smiley *et al.* 2009). These diseases occur most frequently in cool wet soils in areas of higher precipitation but are also important and occasionally severe even in the driest production areas. Although populations of the pathogens are equal in tilled and direct-drill farming systems, a particularly important management practice in direct-drill systems is to equip the harvest combine with a straw and chaff spreader to prevent development of rows of *Pythium*-incited depressed growth where chaff rows have been left when harvesting the preceding crop. Extensive damage occurs without visibly apparent symptoms other than a non-diagnostic lack of vigor and reduced tillering, which are most pronounced in portions of fields with the greatest amounts of crop residue.

Pythium-infected root segments are often lost when roots are washed for inspection, making it difficult to detect these pathogens. The most virulent species in the PNW are *P. ultimum* and *P. irregulare* groups I and IV. A real-time PCR procedure was developed to detect and quantify these species (Schroeder *et al.* 2006). Crop rotation is not useful for controlling *Pythium* diseases but inoculum levels of some but not all species decline during the host-free interval of the wheat-fallow rotation. Partially effective management strategies include planting when soil temperature and moisture are optimal for rapid seedling emergence and establishment, treating seed with an oomycete-specific fungicide, using only recently harvested seed to ensure rapid germination, and controlling the green bridge as discussed for *Rhizoctonia* root rot.

Fusarium crown rot

This disease is very widespread and in the PNW becomes particularly acute on winter wheat planted into cultivated seedbeds before the autumn break, and on spring wheat and barley planted into high-residue seedbeds (Paulitz *et al.* 2002). All of the early work in the PNW was led by Jim Cook, in collaboration with other scientists and farmers. Jim Cook initiated his research soon after training with noted *Fusarium* experts at the University of California at Berkeley and at the University of Adelaide, Waite Campus. The most important crown rot pathogens in the PNW are *Fusarium culmorum* and *F. pseudograminearum*. Because disease incidence is strongly correlated with the quantity of infested residue, practices that enhance soil conservation have led to increasing levels of economic damage from crown rot. Readily identifiable surface residues of previous crops remain for as long as three years in PNW environments that are mostly too dry for decomposition during warm weather and too cold when soil is moist. These features prolong inoculum viability and lead to greater disease severity in direct-drill than cultivated systems.
The most effective management strategy developed

thus far in the PNW is to delay planting winter wheat until the seed-zone temperature falls below 10°C, which is compatible with direct-drill systems but not cultivated fallows that have early sowing dates as the underlying principal for achieving optimal agronomic yield potential. Seed-zone temperature (10-cm depth or greater) can exceed 25°C when wheat is planted early into cultivated fallow using moisture-seeking drills.

Nitrogen management also has a strong impact on crown rot incidence and severity, particularly in low-rainfall environments where plants mature without the benefit of late-season rainfall and where the least expensive fertiliser application strategy is to inject the entire seasonal fertiliser requirement into soil before the crop is planted and before the amount of seasonal precipitation is known. Reducing the stimulation of seedling growth and consequential acceleration of moisture depletion by applying only a portion of the fertiliser before or at planting, and the remainder at some later date, can be used to reduce crown rot severity but this practice may also reduce wheat yield more than the crown rot because it leads to greater late-season moisture competition by winter-annual grass weeds. Wheat cultivars produced for high-protein end uses, such as bread wheat and durum, are particularly susceptible to damage by crown rot because they require a greater amount of applied nitrogen than cultivars produced for low-protein (<10%) end uses. Where both market classes are produced, as in the PNW, growers who apply fertiliser to meet the protein requirements for high-protein wheat almost always have greater losses from crown rot.

Cultivars span the range from susceptible to moderately tolerant, as was first defined by Graham Wildermuth and colleagues at QDPI, Toowoomba. Cultivars with varying levels of tolerance perform, on average, equally against *F. pseudograminearum* in the PNW as in Queensland. However, responses of cultivars are variable over seasons and geographic areas, making it difficult to publish consistently reliable sowing recommendations (Smiley and Yan 2009). Lines from the CIMMYT Root Disease Resistance Nursery coordinated by Julie Nicol (Ankara, Turkey), are being evaluated in an attempt to identify germplasm with improved crown rot resistance. Crosses between the Australian wheats 2-49 and Sunco and several PNW-adapted cultivars have been made in an attempt to improve resistance and especially the stability of that trait over seasons and geographic regions.

Summary

This paper is a tribute Albert Rovira's many contributions to agriculture and to science. His research and his close interaction with practicing farmers and advisors have created many new understandings of the dynamics of root diseases that limit productivity of small-grain cereals, especially in conservation farming systems. Albert's influence has been important throughout the world. This paper summarises selected Pacific Northwest USA research on diseases examined by him, including take-all, *Rhizoctonia* root rot (bare patch), cereal cyst nematode, root-lesion nematode, *Pythium* diseases and *Fusarium* crown rot. In accordance with his current global effort, through the ATSE Crawford Fund, connections were made to exemplify the cross complementation and validation that has occurred for knowledge first gained either in Australia or in North America.

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Rhizoctonia on the Upper Eyre Peninsula, South Australia

N.S. Wilhelm[1]

Abstract

The upper Eyre Peninsula (EP) is a large agricultural region of South Australia dominated by fragile, infertile, calcareous and sandy soils. The rainfall is low and falls mostly during the autumn to spring months, typical of Mediterranean climates. The farming systems are dominated by wheat and barley crops rotated with volunteer pastures consisting of annual *Medicago* and grass species. The pastures are grazed by sheep in low-input management strategies. Rhizoctonia is an endemic problem and appears to flourish in the major environments of this region. While some management strategies can reduce its impact, it is still largely an intractable problem influencing crop productivity, nutrient use efficiency and economic returns. The discovery of biological suppression antagonistic against rhizoctonia disease incidence offers the prospect of removing rhizoctonia as a major production constraint on the upper EP.

The upper Eyre Peninsula

This agricultural region of South Australia produces about one-third of the state's wheat crop (1 MT y^{-1}) and is an area dominated by calcareous, infertile, shallow and sandy soils. The region has a

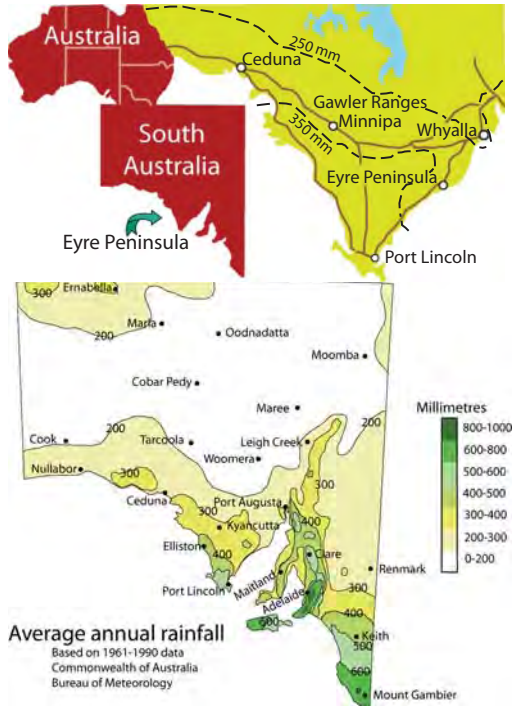

Figure 1. The Eyre Peninsula of South Australia and annual rainfall isohyets in the state (adapted from Anon. 2002)

Mediterranean climate with about 70% of the annual rainfall falling between the months of April and October, or the autumn to spring period. This is the growing season for the farming systems in this region, with the summer being hot and dry (Rovira 1992). Annual rainfall is low and highly variable,

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with the agricultural zone in this region being defined by the 250 mm and 350 mm average annual rainfall isohyets (see Fig. 1).

Nearly all of the farming systems in this region employ a rotation of cereal crops with annual volunteer pastures.

The most frequent combination is two cereal crops (wheat–wheat or wheat–barley) followed by one volunteer pasture of a mix of annual grasses and medic (annual *Medicago* spp.) (Anon. 2002). Sheep in these extensive and low-input management systems are used to graze the annual pastures and cereal stubbles. Long fallows (more than 4 months) are rarely practised on upper EP due to the susceptibility of the sandy soils to wind erosion over the dry months of summer and early autumn. Most crops are established using no-till because this is the most rapid and flexible system for seeding large areas of crop and also maximises the protection of the soil surface from wind erosion (Anon. 2002).

Most of these farms consist of 1500 to 3000 arable hectares that are managed with a low-input, low-cost strategy due to the variable climate and generally low productivity on the shallow and infertile soils. Long-term average wheat yields for the region are about 1.5 t ha^{-1}. Annual stocking rates for sheep are often only 2–3 dry sheep equivalents per hectare (Anon. 2002).

All soil types under agricultural production on upper EP are very old by world standards and hence are highly weathered and leached. This has resulted in widespread and severe infertility in the surface layers of these soils. Many of the essential elements for normal plant growth are in deficient supply in most of the upper EP soils, either due to low inherent levels in the soil matrix, or due to poor availability from the high pH present in most of these soils, or a combination of both factors. Deficiencies of phosphorus, nitrogen and zinc are widespread in crops and pastures while deficiencies of manganese and copper can be severe but often more localised in extent (Reuter 2007). In some of the highly calcareous sandy loams of the upper EP (see Fig. 2), phosphorus deficiency has been endemic, despite the use of granulated high-analysis phosphorus fertilisers. Only fluid phosphorus fertilisers appear to fully correct phosphorus deficiency in these soils and they are not widely used on the upper EP due to their high cost (Holloway *et al.* 2001). Sulphur, potassium, boron

Figure 2. Soil profile of a highly calcareous sandy loam soil in the upper Eyre Peninsula

and sodium are the only essential elements in abundant supply in most upper EP soils. Nitrogen can occasionally occur at abundant levels but only after a vigorous medic-based pasture. However, these occur only sporadically and only following average to wet growing seasons (Anon. 2002).

Break crops for cereals such as canola (*Brassica napus*) or field peas (*Pisum sativum*) are considered to be too unreliable and drought sensitive to be regularly used in rotations on the upper EP and are found only on the wetter fringe of the region, and even then only in limited areas (at most 10% of the cropped area). The medic-based pastures are the only widespread breaks for cereal pests and diseases, but as these pastures frequently have a substantial grass component their value as a cleaning phase for these problems is diminished.

Rhizoctonia (bare patch) on upper EP

In this large and important agricultural region for South Australia, *Rhizoctonia solani* anastomosis group 8 (AG8) causes widespread and frequent damage in all phases of the rotations (Neate and Warcup 1985; Neate 1987).

Figure 3. Rhizoctonia disease patches in a South Australian barley crop

Figure 4. Severe rhizoctonia disease symptoms in an upper EP cereal crop growing on a highly calcareous grey soil

Rhizoctonia solani is a soil-borne fungus whose primary niche in soil ecosystems appears to be as a saprophyte. However, conditions in agricultural systems occasionally occur that appear to be conducive to *R. solani* acting as a pathogen. Under such conditions, the fungus invades the cortex of roots of a wide range of host plants, causing localised lesions and eventually killing the root. In situations where the disease incidence is severe the root system of the host plant can be decimated, leading to poor shoot growth.

Severe stunting in crop and pasture plants appears only in discrete and very clearly defined irregular-shaped patches (see Fig. 3). However, the pathogen (and the symptoms of the disease it causes—brown or absent cortices and 'spear tips' on root ends) can still be found outside these distinct patches (MacNish and Neate 1996). These disease patches often re-occur in the same spots but details of the chemical and biological characteristics specific to these sites, e.g. pathogen inoculum levels, are not fully understood. Patches will be more severe and frequent in barley than in wheat under the same circumstances (Neate 1989).

The disease is generally more severe during the seedling phase of crops and pastures. The severity of the characteristic patches frequently fades as the season progresses into spring; it is unclear why the disease appears to be worse early in plant development.

Rhizoctonia is so widespread across the upper EP that patchy and uneven crops are the norm in many districts (see Fig. 4). The combination of alkaline, sandy, infertile soil types, relatively dry mild starts to each growing season and grass-dominated rotations appears to be very conducive to the development of the disease because grasses in pastures serve as the source of pathogen inoculum in the following crop. It is estimated that as much as $65 million is lost each year through the effect of rhizoctonia in the upper EP (N. Cordon, Primary Industries and Resources of South Australia (PIRSA), pers. comm. 2007), although estimates of economic damage across southern Australia vary widely (Brennan and Murray 1998; Jones 2007).

The conditions that appear to be conducive to the development of the disease in agricultural systems appear to be very complex and dynamic because many attempts to identify the pre-disposing factors have been made and, while some factors have been identified, none have proven to universally explain the epidemiology of the disease.

Cook *et al.* (2008) undertook a comprehensive survey on the upper EP specifically targeting paddocks which farmers rated as either very bad for rhizoctonia or where rhizoctonia was rarely a problem. Detailed analysis of management history and practices followed failed to clearly identify any factor, environment or agricultural practice that was consistently associated with disease severity.

No chemical treatments have yet been found that are both beneficial and cost-effective against rhizoctonia for upper EP farming systems (Cordon 2008). Where soils are sufficiently free of limestone rocks, most farmers will use deep working points (about 5–10 cm below the seed bed) at seeding to cultivate the soil below the seed row. This will reduce rhizoctonia but not eliminate it (Gill *et al.* 2002). Originally, it was thought that this practice physically disrupted the mycelia of *R. solani* and thus its infective potential (Roget *et al.* 1996) but it is now believed that its most important benefit is to foster rapid root growth in the host plant and allow those roots to 'escape' the inoculum-rich upper layers of the soil profile as quickly as possible (Gill *et al.* 2001).

Many farmers on the upper EP will report that the factors listed in Table 1 can influence the disease.

Although the nomination of many of these factors is supported by individual research findings, they do not appear to be universal in their effects.

Despite this long list of do's and don'ts for managing rhizoctonia, the severity and extent of the disease does not seem to be declining except where some farm managers have implemented a continuous cropping enterprise for at least 5–10 years previously. Some of these managers report substantial declines in the severity and frequency of rhizoctonia in their crops. Others, however, with apparently similar systems in similar environments do not report such trends.

However, the discovery of suppression in a similar environment at Avon, in the lower north of South Australia, has introduced a possible solution because it occurred over a range of management systems and rotations (Wiseman *et al.* 1996).

Table 1. Factors that have been identified to influence the incidence of rhizoctonia disease under field conditions. This information is based on multi-year observations in field experiments and farmer paddocks.

Factors that make rhizoctonia worse
• Dry, warm start to the cropping season
• Low fertility, particularly for phosphorus, nitrogen and zinc
• Barley grown rather than wheat
• Cereals grown rather than peas or canola
• Use of sulfonylurea herbicides either with the crop or in prior years
• Seeding systems that do not cultivate below the seed bed
Factors that reduce the severity of rhizoctonia
• Late or wet start to the cropping season
• Adequate or luxury phosphorus and zinc nutrition. High levels of nitrogen fertility or fertiliser applications can help crops 'grow away' from the disease.
• Preceding the cereal crop with either a fallow or canola
• Seeding systems that cultivate below the seed bed or cultivating prior to seeding
• Ensuring any weeds are killed at least three weeks prior to seeding
• Continuous cropping (rather than a ley system of cereal crop – pasture combinations)

Disease suppression against rhizoctonia on the upper EP

Roget (1995) demonstrated that incidences of rhizoctonia disease had disappeared from a long-term rotation trial on a calcareous sandy loam soil in a low-rainfall environment. Research by Wiseman *et al.* (1996) and Roget *et al.* (1999) showed that this suppression was due to a change in the composition and activity of microbial flora and the presence of populations antagonistic to the development of rhizoctonia disease (Barnett *et al.* 2006). Stubble retention and low levels of mineral nitrogen in the soil seemed to be two key factors with this change in the soil microflora.

One of the early questions which flowed from this discovery was whether this could be made to work on the upper EP. Disease suppression active against rhizoctonia has since been identified in a range of cropping environments across southern Australia, including the upper EP (Roget *et al.* 1999; Cook *et al.* 2008; Gupta *et al.* 2009). While the general level of suppression expressed in upper EP soils appears to be lower than at Avon, there are examples of very strong suppression in some situations.

We are still not sure how to manipulate systems to improve the level of suppression expressed, especially in the low-productivity environments of the upper EP. Roget and Gupta (2006) reported that the expression of disease suppression can be modified by changes in the C and N turnover, par-

ticularly over summer and autumn—for example, a narrow C:N ratio could switch off the suppression. However, other aspects about the management of suppression are still not clear, except that it appears that the suppressive microflora are often limited by the availability of simple carbon substrates (e.g. carbohydrates) in the low-rainfall cropping environments in SA.

Future prospects

Suppression does not appear to operate strongly on the highly calcareous grey sandy loams of the upper EP. This is an environment where rhizoctonia is widespread, most severe and most frequent. It is also an environment where productivity is very low (and hence carbohydrate supply for soil microflora is poor), soils are often dry and highly aerated (due to their very high infiltration rates and the low rainfall in the area) and where phosphorus deficiency is severe, widespread and frequent. Crops grown on this soil type are almost always phosphorus deficient, regardless of the phosphorus fertiliser regime (unless it involves fluid phosphorus) (Holloway *et al.* 2001).

Phosphorus deficiency weakens defence pathways in plants and reduces productivity. Reduced productivity reduces food supply to soil microflora, including suppressive communities. Unfortunately a potential pathogen with strong saprophytic capabilities can compete particularly well in such an impoverished environment.

Could phosphorus nutrition management be the key to fostering suppression on the upper EP and unlocking the solution to rhizoctonia as an intractable problem?

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Influence of carbon input to soil on populations of specific organisms linked to biological disease suppression of *Rhizoctonia* root rot

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Abstract

Labile soil organic carbon has been suggested as one of the main drivers of biological disease suppression, both in terms of increasing populations of suppressive organisms as well as providing an energy source for these organisms to function. Eyre Peninsula (EP) soils in South Australia generally have relatively low soil organic carbon (<1.5%) which may preclude the development of biological disease suppression. It was hypothesised that increased input of organic carbon to these soils could foster the development of biological disease suppression. A controlled environment experiment was undertaken to investigate the effect of two types of carbon inputs (wheat stubble and young wheat roots) to an EP soil on the populations of organisms linked to biological disease suppression and on disease severity of root rot caused by *Rhizoctonia solani* AG-8. After a six-month incubation period, a sub-sample of soil from each treatment was used for quantification of soil organisms potentially involved with suppression (*Pantoea agglomerans*, *Exiguobacterium acetylicum*, *Microbacterium* sp. and *Trichoderma* sp.). The remaining soils were then used in a pot bioassay to measure disease severity on wheat seedlings after inoculation and incubation with *Rhizoctonia solani* AG-8. Results showed that carbon input increased the quantity of DNA of potentially suppressive organisms; young root carbon > stubble carbon > control for *Pantoea agglomerans*, *Exiguobacterium acetylicum* and *Microbacterium*, whilst for *Trichoderma* groups the increase in amounts of DNA was determined by the type of carbon input and the *Trichoderma* group. Overall, input of either source of carbon (stubble or young roots) to this EP soil reduced wheat seedling infection by *Rhizoctonia solani* AG-8 in a pot bioassay.

Introduction

The adoption of conservation tillage farming systems and direct drilling in recent years appears to have increased the severity and extent of *Rhizoctonia* root rot caused by *Rhizoctonia solani* AG-8 in Australia (Rovira 1986). The Eyre Peninsula (EP) in South Australia (SA) is a region which produces about 40% of the SA wheat crop but is particularly vulnerable to *Rhizoctonia* infestation due to edaphic and climatic constraints (Coventry *et al.* 1998). Indeed, it has been estimated that *Rhizoctonia* root rot could reduce the yield in the northern part of this region by up to 60% (Crouch *et al.* 2005).

Roget (1995) reported the decline of *Rhizoctonia* root rot at a long-term trial site at Avon in the mid-north of SA. Further investigations demonstrated that this was due to a biological factor within the soil (Wiseman *et al.* 1996). More recent work has lead to isolation of specific soil organisms from the

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soil at this site that act in combination to reduce disease severity of *Rhizoctonia* root rot (Barnett 2005; Barnett *et al.* 2006). It has also been reported that disease suppression is a function of the population, activity and composition of a diverse microbial community and that an increase in labile carbon substrates is one of the main drivers behind biological disease suppression of *Rhizoctonia* root rot (Roget *et al.* 1999; Gupta *et al.* 2007).

Since soils from the EP generally have low amounts of labile (biologically available) carbon (Coventry *et al.* 1998; McNeill *et al.* 2001), it was hypothesised that addition of an organic carbon source to these soils could lead to a reduction in *Rhizoctonia* root rot via biologically mediated disease suppression. Thus a study was undertaken to investigate if:

- the addition of two carbon types of sources representative of the two main types of inputs of carbon in agricultural systems (young roots which have a narrow C : nutrient ratio or cut stubble with a wide C : nutrient ratio) to a selected EP soil would result in lower disease severity
- these different carbon sources reduced disease severity by different levels
- the reduced disease severity was correlated with amounts of DNA for specific organisms linked to reducing disease severity in another soil in SA.

Materials and methods

A soil previously identified as non-suppressive to *Rhizoctonia* (Cook *et al.* 2007) was collected in March 2007 from the top 10 cm depth in a 200 m transect across a farmer paddock on the Eyre Peninsula at Mount Damper (33°04.159′S, 135°03.584′E), air dried and sieved through a two-millimetre sieve. The soil was non-calcareous (1.34% $CaCO_3$) with a $pH_{(CaCl_2)}$ of 7.6, an organic carbon of 1.4% and a CEC of 19.14 meq kg^{-1}.

Twelve plastic containers (31 cm × 13 cm × 12 cm) each holding 3.5 kg of air dried soil and watered by weight to 75% of field capacity were prepared. Three experimental treatments were then applied—addition of young root carbon, stubble carbon or no carbon (control). The addition of young root carbon (Treatment 1) was obtained by planting a high density (875 seeds m^{-2}) of surface-sterilised wheat seeds (*Triticum aestivum*, cv. Yitpi) into each of the four containers filled with soil and growing plants for 28 days (Mazzola and Gu 2000; Miche and Balandreau 2001; Barnett *et al.* 2006). After 28 days plant shoots were cut off just above the soil level and then the root-soil mixture in each container was broken up and mixed well. This was repeated three times resulting in a total of four cycles for the growing wheat plants. The stubble carbon input (Treatment 2) was set up by mixing chopped (about 1 cm long) wheat stubble into four of the containers of soil (5.8 g stubble kg^{-1} dry soil) at the same time as the root treatment was initiated. After 28 days (length of a cycle for Treatment 1) the soil and stubble mix was mixed in each container, and this was repeated for a total of four cycles. No carbon was added to the containers in the control treatment but the soil in each container was mixed well every 28 days at the same time as for treatments 1 and 2. For practical reasons, after four growing cycles, the soil from treatments in each container was air-dried before use in the bioassay.

A sub-sample of air-dry soil was removed from each container and sent to the SARDI Diagnostics Group (South Australian Research and Development Institute, South Australia), for quantification of DNA from the pathogen *Rhizoctonia solani* AG-8 (Whisson *et al.* 1995) and the beneficial organisms *Exiguobacterium acetylicum*, *Pantoea agglomerans*, *Microbacterium*, *Trichoderma* Group A, (sect. *Pachybasium*) and Group B (sect. *Trichoderma* (see Fig. 2) described by Kullnig-Gradinger *et al.* 2002).

The bioassay was based on methods described by Barnett *et al.* (2006). Briefly, in this work 300 g dry soil at 75% of field capacity was weighed into three bioassay pots from each container. Each pot was inoculated at half the pot height (5 cm) with three, 10-mm full strength potato dextrose agar plugs infested with *R. solani* AG-8. Seven surface-sterile wheat seeds were planted and thinned to five seedlings per pot and after 28 days growth in a controlled environment room (15°C and 12 hour day–night regime), plants were sampled, root washed and the disease incidence was measured by counting the number of seminal roots truncated by *Rhizoctonia* root rot before 10 cm (height of the 300 ml pot) and those infected but not truncated. These counts were used to calculate percentage root infection:

Percentage root infection = (No. truncated roots + (No. infected roots / 2) × 100) / (Total no. seminal roots)

Data were analysed as ANOVA, RCBD using GenStat Eighth Edition. Least significant difference (lsd) at $P = 0.05$ was used for comparison of treatment means.

Results

Rhizoctonia solani AG-8 DNA amounts for soil from the treatments prior to the bioassay were higher ($P = 0.05$) in the soil with young root carbon treatment (3.12 $\log_{(10)}$ pg DNA g^{-1} soil) than in either the stubble carbon or control treatments (0.19 and 0.52 $\log_{(10)}$ pg DNA g^{-1} soil, respectively).

In the bioassay, percentage root infection was lower ($P = 0.05$) in both the carbon input treatments compared to that of the control (Fig. 1).

Amounts of DNA ($P = 0.05$) from both root associated bacteria *Exiguobacterium acetylicum* and *Pantoea agglomerans* were higher in the young root carbon treatment (Fig. 2). The amount of *Pantoea agglomerans* DNA in the stubble carbon treatment was higher than that in the control treatment (Fig. 2).

The amount of DNA for *Microbacterium* increased in the order control < stubble carbon < young root carbon addition treatment ($P = 0.05$) (Fig. 3).

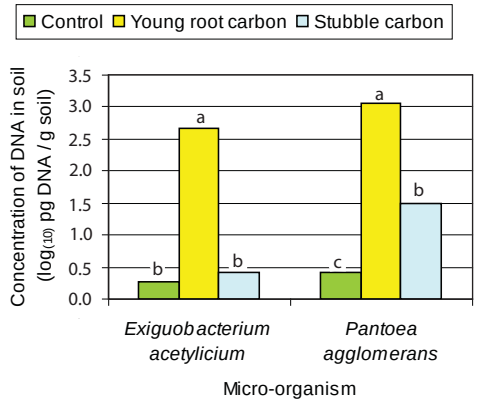

Figure 2. Amounts of DNA of *Exiguobacterium acetylicum* ($lsd_{(0.05)} = 0.08$) and *Pantoea agglomerans* ($lsd_{(0.05)} = 0.72$) extracted from Mount Damper soil which had been pre-treated for six months with a carbon addition treatment (young root or stubble) or left untreated (control). Different letters show significant differences at $P = 0.05$ between treatments within each organism group only.

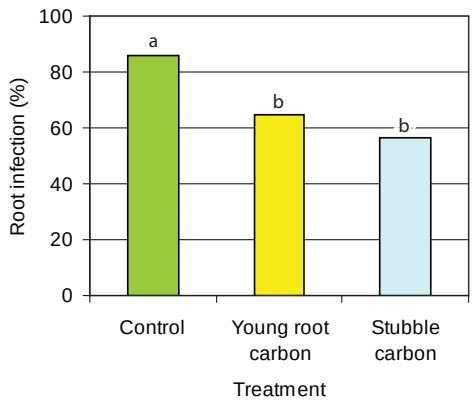

Figure 1. Percentage seminal root infection ($lsd_{(0.05)} = 11.19$) for wheat plants grown in a bioassay of Mount Damper soil which had either been pre-treated for six months with a carbon addition treatment (young root or stubble) or left untreated (control). All pots were inoculated with *Rhizoctonia solani* AG-8. Different letters indicate significant differences at $P = 0.05$.

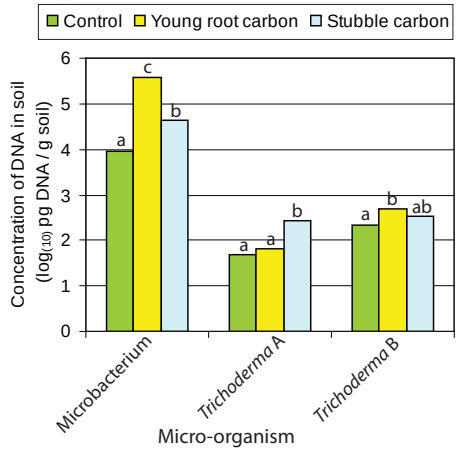

Figure 3. Amount of DNA of *Microbacterium* ($lsd_{(0.05)} = 0.14$), *Trichoderma* group A ($lsd_{(0.05)} = 0.28$) and *Trichoderma* group B ($lsd_{(0.05)} = 0.27$) from Mount Damper soil which had been pre-treated for six months with a carbon addition treatment (young root or stubble) or left untreated (control). Different letters show significant differences at $P = 0.05$ between treatments within each organism group only.

Addition of stubble carbon resulted in the highest amount of DNA for *Trichoderma* group A (P = 0.05) whilst for *Trichoderma* group B, the young root carbon treatment had the higher amount of DNA (P = 0.05) (Fig. 3).

Discussion

In a controlled environment experiment, amendment with two different carbon inputs (young roots or stubble) resulted in less disease than the unamended control treatment, and the amount of DNA for the organisms quantified tended to be highest in the young root carbon treatment and lowest in the unamended control.

In this work, the young root carbon treatment had significantly more DNA for *Exiguobacterium acetylicum* and *Pantoea agglomerans*, which are both root-associated plant growth promoting bacteria linked to biological disease suppression at Avon (Barnett *et al.* 2006). Since the young root carbon treatment involved four cycles of growing roots, it is not surprising that this treatment had such large amounts of DNA for these organisms.

Although these organisms do not act alone in the expression of biological disease suppression (Barnett *et al.* 2006), they are likely to have contributed to the decrease in root infection. *Microbacterium* spp. are soil-associated organisms (Barnett *et al.* 2006) that had the greatest amount of DNA in the young root carbon treatment, followed by slightly less in the stubble treatment, whilst the lowest amount was in the unamended control. The difference between carbon treatments is perhaps due to the young root material being more decomposable, with low-molecular-weight carbon compounds readily available for soil organisms, than the stubble which tends to have high-molecular-weight carbon compounds and is less decomposable with a wide C : nutrient ratio (e.g., C:N of 100:1, Tiessen *et al.* 1984).

The triplet combination of *Microbacterium*, *Exiguobacterium acetylicum* and *Pantoea agglomerans* have been reported to act effectively to decrease disease severity of *Rhizoctonia solani* under controlled environment (Barnett *et al.* 2006). Our results supports this concept, that is, greater amounts of DNA from all three of these bacteria in the carbon-amended soils than in the control treatment, which had the least amount of DNA from these organisms and the highest root infection.

For both of the *Trichoderma* groups quantified in this work, the unamended control treatment had the least amount of DNA for *Trichoderma* and the most root infection. A number of *Trichoderma* spp. have been well documented as biocontrol agents (Harman *et al.* 2004); they may also have contributed to the decrease in disease in the carbon-amended treatments in this work.

Despite an improved ability over the past decade to quantify some of the organisms linked to specific functions such as disease suppression, it is important to note that many other organisms are likely to have been involved in decreasing disease incidence, especially in the field environment. This present work has highlighted the importance of considering multi-organism whole–system interactions to understand the influence of added carbon substrates on *Rhizoctonia* disease incidence. Another consideration is that an amount of DNA does not necessarily equate to a measure of organism activity, so it is possible that the organisms may be present but not necessarily functioning and able to cause suppression, although the reduction in disease incidence in this work suggests otherwise. Nevertheless, it is acknowledged that further work is required to more fully define the functional ecology underlying the biological suppressive capacity of soils in the field environment.

Conclusions

Under controlled environment conditions relatively large inputs of carbon in the form of young roots or wheat stubble to a soil from EP, SA, resulted in a decreased severity of *Rhizoctonia* root rot as well as an increase in populations of specific organisms suggested to be linked to soil-borne disease suppression. Given the widespread adoption of no-till as a management practice on the EP, where stubbles are retained and there is minimal soil disturbance, the increased C inputs should therefore increase the potential for development of biological suppression to soil-borne diseases. However, mechanisms for an increase in the severity of *Rhizoctonia* root rot under no-till and the development of disease suppression under some regional climatic and edaphic constraints to agricultural production are not completely understood and require further investigation.

Acknowledgements

Thank you to GRDC for funding RSD's scholarship and GRDC, SARDI and SAGIT for supporting the research. Thank you to A. Cook for the use of her data, W. Sheppard for collecting all the soil and S. Anstis for the *Rhizoctonia*. Thank you also to N. Wilhelm, K. Clarke and C. Heddles for their support, discussions and advice. Lastly thank you to K. Ophel-Keller, A. McKay and all the other staff at SARDI diagnostics centre for DNA analysis on these soils and to SAGIT for funding the development of these tests.

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Minimum tillage and residue retention enhance suppression of *Pratylenchus* and other plant-parasitic nematodes in both grain and sugarcane farming systems

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Abstract

Observations in sugarcane cropping soils in Australia showed that conserving soil organic matter through practices such as residue retention and minimum tillage improved a range of soil physical and chemical parameters including total C, labile C, aggregate stability, water infiltration rate and cation exchange capacity, and reduced bulk density and surface crusting: all parameters that are positively associated with soil and plant health. Results of field and microplot experiments indicated that biological mechanisms that suppressed plant-parasitic nematodes were also enhanced. *Pratylenchus zeae* and *Meloidogyne javanica* did not multiply as readily when soils received continual C inputs from a mulch layer of plant residue, were not disturbed by tillage, or were amended with high C/N residues. Numerous suppressive mechanisms are probably involved, but predatory fungi that obtain N from nematodes in N-limited environments appear to be contributing. In cereal-growing soils under minimum tillage, populations of *Pratylenchus thornei* were relatively low in the upper 25 cm of the soil profile and remained low when surface soils were potted and planted to wheat, indicating that the nematode did not multiply readily when stubble was retained on the soil surface and C levels were relatively high. Thus in both sugarcane and cereal-growing soils, minimum tillage and residue retention appear to play a role in improving the suppressiveness of soils to plant-parasitic nematodes.

Introduction

In the subtropical and tropical regions of north-eastern Australia, grain crops (predominantly wheat, sorghum and chickpea) and sugarcane are the main cropping industries. The grain-growing region occupies about 4 million hectares and extends from just south of Tamworth in New South Wales (~32°S) to near Emerald in Queensland (~23°S). Sugarcane is grown on about 440 000 ha of land along the east coast, from Grafton in northern New South Wales to just north of Cairns in Queensland.

Grain crops and sugarcane have been grown in this area for more than 100 years, and for most of that time land was cultivated with conventional tillage implements before crops were planted. However, the grain industry began to change to reduced tillage in the 1980s, with zero tillage and residue retention now standard practice throughout the industry. Following research demonstrating the benefits of a farming system based on a legume rotation crop, controlled traffic with GPS guidance, minimum tillage and residue retention (Garside *et al.* 2005), a similar change is now taking place in the sugar industry.

Plant-parasitic nematodes are important pests of both grain crops and sugarcane, and this paper uses experimental data that has been published else-

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where (Stirling 2007, 2008) to examine the impact of these recent changes in farming systems on nematode populations. Tillage and residue management affect the rate of accumulation of soil organic matter (Franzluebbers 2004) and since organic matter plays a key role in inducing suppressiveness to a wide range of soilborne pathogens, including nematodes (Stirling 1991; Akhtar and Malik 2000; Stone *et al*. 2004), it was considered that suppressive mechanisms against plant-parasitic nematodes were likely to be enhanced by a move towards minimum tillage and residue retention.

Experiments with sugarcane soils

Field experiment

The first observations on the effect of tillage on nematode population dynamics were made in a field experiment established at Bundaberg in 2003. Treatments included various lengths of bare fallow, grass and legume breaks, and some organic amendments (Bell *et al*. 2006), but those of interest from a nematological perspective are listed in Table 1. The various breaks had expected effects on populations of *P. zeae*: the nematode population density at the time of planting sugarcane (Pi) declined as the length of the bare fallow increased, the legume break reduced nematode populations to very low levels relative to continuous sugarcane and the grass pasture maintained moderate populations of nematodes (Table 1). There were marked differences, however, in the way lesion nematode populations responded when sugarcane was replanted. Nematode multiplication rates in the first 13 months after planting were much higher in fallowed soil than in soil that had been cropped, probably because biological activity had declined markedly in the fallow (Bell *et al*. 2006). Although differences in final population densities (Pf) were not always significant, the nematode did not multiply as readily in non-tilled soils and the final nematode population in the non-tilled legume treatment was one-third as high as the 30-month bare fallow treatment, despite the fact that Pi in both treatments was similar at planting.

Table 1. Effect of rotation and tillage treatments on population densities of *Pratylenchus zeae* at the time sugarcane was planted (Pi), and 13 months after planting (Pf). Modified from Bell *et al*. 2006.

Treatment	Tillage	Number of *P. zeae* /200 ml soil	
		Pi	Pf
30 month bare fallow	+	1 a	1103 a
12 month bare fallow	+	43 b	1224 a
6 month bare fallow	+	81 b	729 ab
Continuous sugarcane	+	285 c	777 ab
30 month grass pasture	+	81 b	478 ab
30 month grass pasture	–	125 b	315 b
30 month legume crop	+	1 a	958 ab
30 month legume crop	–	1 a	378 ab

Means within a column followed by the same letter are not significantly different ($P = 0.05$)

Pot experiment

The above result suggests that lesion nematode multiplication rates are influenced by the background soil biology and that disturbance caused by the conventional tillage practices used during the planting operation may be one of the reasons that nematodes rapidly re-infest sugarcane after it is replanted. This hypothesis was tested by comparing nematode multiplication in pipes 20 cm long and 10 cm diameter filled with soil from two sugarcane fields, one with sandy loam soil and the other with sandy clay loam soil. Pipes were hammered into the soil surface and the soil cores were then left undisturbed, or the soil was sterilised by autoclaving or disturbed by periodically mixing it by hand. Sugarcane was planted in ten replicate pipes of each treatment and then *P. zeae* was added to half the pipes so that the initial inoculum density in those pipes was similar in all treatments (about 250 nematodes/200 mL soil). In another experiment with root-knot nematode (*Meloidogyne javanica*), all inoculated pipes received the same number of nematodes (about 130 second-stage juveniles/200 mL soil). The results of the first experiment (Table 2) showed that *P. zeae* multiplied best in autoclaved soil and least in undisturbed soil. In pipes that were not inoculated, the final nematode population was much higher in disturbed than undisturbed soil, despite the fact that the initial nematode density was reduced by 30% and 70% in the disturbed soils because of nematode mortality during the mixing process. In inoculated pipes, populations of *P. zeae* increased about 11-fold in autoclaved soil, whereas there were only 5-fold and

Table 2. Final nematode population densities in two experiments where sugarcane was grown in pipes filled with autoclaved, disturbed or undisturbed soil that was either inoculated with 250 *Pratylenchus zeae*/200 mL soil (experiment 1) or 130 *Meloidogyne javanica*/200 mL soil (experiment 2). (G.R. Stirling, unpublished data).

Soil treatment	Experiment 1 No. *P. zeae*/200 mL soil[A]		Experiment 2 No. *M. javanica*/200 mL soil[A]	
	Inoculated	Not inoculated	Inoculated	Not inoculated
Autoclaved	2817 a	0 d	285 a	0 c
Disturbed	1276 b	1663 ab	157 a	0 c
Undisturbed	514 c	464 c	20 b	0 c

[A] Nematode numbers are means for two soils, and are back-transformed means of transformed data [$\log_{10}$ (no. nematodes+1)].
Within each experiment, numbers followed by the same letter are not significantly different ($P = 0.05$)

2-fold increases in disturbed and undisturbed soil, respectively. In the second experiment, *M. javanica* was detected only in inoculated soils, with final nematode numbers significantly higher in autoclaved and disturbed soil than in undisturbed soil (Table 2).

Experiments with grain-growing soils

In four successive years (2003–2006), soil from a depth of 0–25 cm was collected in April from farms in the Goodiwindi–North Star region of Queensland/northern NSW and from the Darling Downs (Jimbour–Macalister) region of Queensland. Soil was obtained from fields that were managed under the standard zero-till cropping system and also from areas under permanent pasture or native vegetation dominated by brigalow (*Acacia harpophylla*). Treatments imposed on soils included gamma irradiation (25 kGy), heat (65°C for 12 hours) and a range of physical and chemical treatments that were likely to affect the soil biology. Broadly similar methods were used for each experiment, with 1.5 L pots being filled with treated or untreated soil and planted with a cultivar of wheat (Sunvale) that is susceptible to but tolerant of *Pratylenchus thornei*. In cases where treatments involved the addition of *P. thornei*, the population density was adjusted to about 1.5 nematodes/g soil by adding the appropriate number of nematodes so that initial inoculum densities were similar in all treatments. Exceptions included some cropped soils where the nematode population density was already > 5 *P. thornei*/g soil. Final nematode population densities were determined by extracting nematodes from a mixture of soil and roots after plants had grown to maturity (about 110 days). Brief details of the design of each experiment are:

2003: 2 soils (with a history of either cropping or pasture) × 2 soil depths (0–5 and 5–15 cm) × ± heat × ± *P. thornei* × 5 replicates.

2004: 2 soils (with a history of either cropping or pasture) × 16 treatments (including untreated, heat, gamma irradiation, metham sodium, freezing, mancozeb and organic amendments) × 4 replicates.

2005: 21 soils with different histories × untreated soil or gamma-irradiated soil × ± *P. thornei* × 3 replicates.

2006: 3 cropped soils from different farms × 3 treatments (gamma irradiated soil, gamma irradiated soil + 10% untreated field soil and untreated field soil) × ± *P. thornei* × 5 replicates.

Results of these experiments indicated that when a single wheat crop was grown in untreated field soil, populations of *P. thornei* generally increased 2–4 times. However, when the soils were sterilised by heat, gamma irradiation or fumigation, there was a 5–21 fold increase in nematode populations. In sterilised soil to which *P. thornei* had been added, final nematode population densities were 20–40 *P. thornei*/g soil, whereas they were generally only 3–10 *P. thornei*/g in soil from crop, pasture or native vegetation sites, despite the fact that similar numbers of nematodes were present at planting. Results from the 2005 experiment provide an indication of the data obtained (Table 3).

Discussion

A new farming system based on legume rotation crops, controlled traffic using GPS guidance, minimum tillage and residue retention has recently been introduced into the Australian sugar industry. The soil health improvements obtained from this system

Table 3. Final population densities of *Pratylenchus thornei* on wheat in field soil, and in field soil or gamma-irradiated soil, after *P. thornei* was added to achieve an initial inoculum density of 1.5 nematodes / g soil. Data from an experiment with soils collected in 2005.

Location of soil samples	Land use	No. *P. thornei* / g soil		
		Field soil (no *P. thornei* added)	Field soil + *P. thornei*	Gamma irradiated soil + *P thornei*
Billa Billa	Cropping	0.3	5.8	36.9
Jandowae	Cropping	0.1	3.3	24.3
Jimbour	Cropping	0.5	0.7	22.9
North Star	Cropping	0.2	13.3	28.0
Yelarbon	Cropping	2.2	8.8	30.0
North Star	Brigalow	0.8	5.9	30.7
Jandowae	Pasture	0.1	4.6	13.7
Jimbour	Pasture	0.2	7.8	23.4
LSD (P = 0.05)			10.4	

(higher soil C, better aggregate stability, higher rainfall infiltration rates, increased cation exchange capacity, greater amounts of potentially mineralisable N and reduced bulk density and surface crusting) have been discussed in detail elsewhere (Stirling 2008). An additional soil health benefit, however, is a reduction in problems caused by plant-parasitic nematodes. Stirling *et al*. (2001, 2007) clearly showed that including a legume rotation crop in the farming system reduced the population density of key nematode pests, while other studies (Stirling *et al*. 2005; Stirling 2008) have shown that organic inputs from mulches and amendments play a key role in enhancing suppressiveness to these pests. The three experiments presented in this paper provide evidence that benefits are also obtained from minimum tillage and residue retention. Root-knot and lesion nematodes, the most important nematode pests of sugarcane, did not multiply as readily when crop residues were retained on the soil surface and the soil was not cultivated or disturbed before planting.

The finding that *P. thornei* did not multiply readily in potted soils from the northern grains belt was unexpected, given that the nematode is widely distributed in this region and is often found at very high population densities in grain-growing soils (Thompson *et al*. 2008). The result was not so surprising, however, when sampling depth is considered. All soils were collected from a depth of 0–25 cm and previous work has shown that populations of *P. thornei* in the field are generally lower near the surface than at depth.

Although the reasons for relatively low nematode multiplication rates in the soils used in this study have not been fully explored, differences in the amount of root biomass available to nematodes may explain some of the effects observed. In the field, sugarcane shoots emerge more slowly in minimum-tilled plots than following cultivation, and since this probably influences root biomass, nematode multiplication in non-tilled plots in the field experiment may have been limited by the amount of food available to the nematode. However, in the pot experiment, differences in nematode multiplication between disturbed and non-disturbed soils were not due to differences in plant growth. In the pot experiments with wheat, plants generally grew better when soil was sterilised but this was not the sole reason for increased nematode multiplication rates in heat-treated, gamma-irradiated and fumigated soils. Final nematode population densities and plant biomass were not strongly correlated in any experiment, nematode multiplication rates remained relatively high when shoots of plants growing in sterilised soil were cut back to limit biomass production, and the nematode population densities achieved in untreated soil were well below the carrying capacity of the potted root systems.

A likely explanation for most of the observed effects is that biological control mechanisms are operating in sugarcane and grain-growing soils and they play a role in regulating nematode populations and affecting nematode multiplication rates. These suppressive forces appear to be particularly active in situations where residues are retained on the soil

surface and minimum tillage is a component of the farming system. Since soil near the surface is more suppressive than soil at depth and most sugarcane and cereal roots are found in the upper 25 cm of the soil profile, these biological buffering mechanisms are likely to be important from a practical perspective. Plant-parasitic nematodes cost the sugarcane and wheat industries $82 million and $69 million, respectively, in northern Australia (Blair and Stirling 2007; Thompson *et al*. 2008) and so any reduction in nematode populations in the zone where most root biomass is located is likely to result in major economic benefits.

To date, no attempt has been made to determine which components of the soil food web are contributing to these suppressive effects. Numerous parasitic and predatory organisms are probably involved, but fungi that prey on nematodes in high-C, low-N environments may be important in sugarcane soils (Stirling *et al*. 2005). Other parasites and predators of nematodes that have been found in sugarcane soils include two fungal parasites of nematode eggs (*Pochonia chlamydosporia* and *Paecilomyces lilacinus*), a bacterial parasite (*Pasteuria*) that has host-specific strains capable of attacking *Pratylenchus* and *Meloidogyne*, and a wide range of dorylaimid and mononchid predators of nematodes. Little is known about mechanisms of suppression in grain-growing soils, but the fact that high multiplication rates in sterilised soil were largely reversed by adding 10% field soil (Stirling 2007) suggests that microbial antagonists are involved rather than larger predatory organisms.

One clear message that emerges from these studies is that soil C plays a key role in biological suppression of nematodes. Since some of the organisms likely to be involved have a saprophytic phase and many parasites and predators use bacterial and fungal-feeding nematodes as a food source, the amount of biological control that occurs in a soil will be dependent on the quality and quantity of resources that sustain the soil food web. It is therefore reassuring that practices which increase C inputs (e.g., retention of residues, introduction of pasture leys or high-residue rotation crops and the use of waste products such as mill mud as amendments) or limit losses of soil organic matter (e.g., reduced tillage) are becoming an increasingly important component of both sugarcane and grain farming systems.

Acknowledgements

The sugarcane work was funded by SRDC through the Sugar Yield Decline Joint Venture, while the grains work was funded by GRDC through its Soil Biology Initiative.

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N.P.E. Reddy

Variability among the isolates of *Sclerotium rolfsii* (Sacc.) causing stem rot of peanut (*Arachis hypogaea* L.)

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Abstract

Peanut is an important oilseed crop in India, covering half of the area under oil seed production. The crop is affected by a soil-borne pathogen *Sclerotium rolfsii* causing stem rot. Roving survey results revealed an average 20% incidence of the disease in the Rayalaseema region of Andhra Pradesh, India. Twenty isolates of *S. rolfsii* were collected and subjected to variability studies. Variability has been observed in growth on PDA medium, sclerotial size, colour, pathogenicity on peanut, and RAPD profiles. Among all the isolates CSr 2, 3 and 4, and KSr 12, 14, 15, 16, 17 and 18 have shown maximum growth (90 mm) and least growth was observed in case of KSr 19 isolate (62.7 mm) on PDA medium. The isolate KSr 18 produced the most sclerotia (571/plate) and isolate CSr 6 the least (30/plate). The sclerotia were largest (2.2 mm) in the case of isolate KSr 16 and smallest (0.90 mm) for isolate CSr 4. The colour of sclerotia varied from light brown to reddish-brown to dark brown. Isolates CSr 4, KSr 19 and KSr 20 were highly virulent, with 100% disease incidence on peanut variety TCGS-888 in red loamy soil in pot culture studies. The ITS amplified region of rDNA produced a fragment of about 650–700 base pairs (bp) which confirms the genus *Sclerotium*. RAPD profiles with random primers, viz. OPA-01, OPA-12, OPA-17, OPA-18 and OPA-20 reflected the genetic diversity among the isolates with the formation of two clusters. Cluster I consisted of four isolates, viz. CSr 9, KSr 17, CSr 1 and KSr 19 of which the first two and last two form two separate sub-groups Ia and Ib. Cluster II contained four isolates, viz. CSr 2, CSr 10, KSr 15 and KSr 20. The primer OPA-01 amplified two unique bands of about 100 bp and 250 bp in the case of the highly virulent isolate KSr 19, which can be used for the development of species-specific SCAR (sequence characterised amplified region) markers, whereas the primer OPA-12 amplified specific bands of 1400 bp and 1500 bp for the less virulent KSr 15 isolate. However, the ITS-RFLP results with *Alu*I, *Hinf*I and *Mse*I enzymes did not show any polymorphism among the isolates under study. This work improves our understanding of the epidemiology of the pathogen *S. rolfsii* and complements our effort to develop control options that reduce disease incidence and yield losses in the economically important peanut crop in southern India.

Introduction

Groundnut or peanut (*Arachis hypogaea* L.) is a major legume and important oil seed crop in southern India. In India it is grown over an area of 6.74 million ha with an annual production and productivity

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of 7.99 million t and 1185 kg ha^{-1}, respectively. In Andhra Pradesh it is grown on up to 1.87 million ha with 1.64 million t of production and a productivity of 728 kg ha^{-1} (Directorate of Economics and Statistics, Hyderabad 2005–2006).

In peanut, many diseases occur at different growth stages. Among these, stem rot caused by *Sclerotium rolfsii* Sacc. (teleomorph *Athelia rolfsii* (Curzi) Tu and Kimbrough) is one of the important diseases. It causes severe damage to the crop, and yield losses to an extent of 25% have been reported (Mayee and Datur 1988). Analysis of variability within pathogen populations helps to understand host-pathogen co-evolution and epidemiology, and for developing strategies for resistance management (Castro *et al*. 2000; Morris *et al*. 2000).

The internal transcribed spacer (ITS) analysis of rDNA is a reliable method for taxonomic species identification and random amplified polymorphic DNA (RAPD) for determining sub-population diversity within the species (Freeman *et al*. 2000). The aim of this study was to determine the variability in cultural and morphological properties, pathogenicity and genetic diversity among the isolates of *S. rolfsii* from peanut-growing regions of Andhra Pradesh, India.

Materials and methods

Survey, isolation and identification of pathogen

A roving survey was conducted in Chittoor and Kadapa districts of the Rayalaseema region, Andhra Pradesh, to estimate disease incidence and to collect peanut plants infected with *Sclerotium rolfsii*. During the survey, twenty plant samples were randomly collected and the pathogen was isolated from infected peanut plants using the tissue segment method (Rangaswami and Mahadevan 1999). Isolates were maintained on potato dextrose agar (PDA) by periodical transfer (Dhingra and Sinclair 1995) and identified using standard mycological keys (Barnett and Hunter 1972).

Cultural and morphological variability

Cultural and morphological variability was evaluated on potato dextrose agar (PDA) medium. A mycelial disc 5 mm in diameter was taken from the edges of actively growing culture and inoculated in the centre of a plate and incubated at 28 ± 2°C. Radial growth was measured at 24 h intervals until the entire plate was covered with the fungus, measuring along the diameters at right angles and then averaging the data. There were three replications for each isolate. Morphological characteristics of sclerotia, viz. time taken for sclerotial production, size, colour and number of sclerotia produced were recorded for each isolate. Sclerotial size was measured using an image analysis software program with a Motic Camera (USA). The size of 30 sclerotia was measured in each replication and the results were then averaged.

Pathogenic variability

The pathogenic potential of each isolate of *S. rolfsii* was tested on TCGS-888 peanut variety in a pot culture experiment. For artificial inoculation, dried sclerotia were germinated for 36–48 h on PDA at 28°C and a 1-cm disc of germinated sclerotia along with actively growing mycelium was transferred to the stem base and covered with coarse sand up to a height of 1.5 cm and maintained in a moist condition (Shokes *et al*. 1996). Seedling mortality was recorded 15 days after inoculation (DAI). There were three replications for each treatment.

Percent disease incidence (PDI) was calculated using the following formula:

$$\text{PDI} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

Genetic diversity among *S. rolfsii* isolates

Extraction of fungal genomic DNA

Eight isolates of *S. rolfsii*, viz. CSr 1, CSr 2, CSr 9, CSr 10, KSr 15, KSr 17, KSr 19 and KSr 20, differing in degrees of pathogenicity, were selected for molecular characterisation using RAPD, ITS-PCR and ITS-RFLP methods. The total genomic DNA of *S. rolfsii* isolates was extracted from vegetative mycelium using the procedure described by Murray and Thompson (1980) with minor modifications.

PCR amplification for RAPD analysis

Five different random primers belonging to the Operon 'A' series, viz. OPA-01, 12, 17, 18 and 20 (Operon Technologies Inc.), were used to detect polymorphism among the isolates. PCR amplifications were carried out in 0.2 ml Eppendorf tubes with 25 µl reaction mixture (2.5 µl of 10x Taq buffer, 2.5 µl of 25 mM $MgCl_2$, 2.5 µl of primer (1 picomole μl^{-1}), 0.5 µl of 100 mM dNTP mix, 0.2 µl of *Taq* polymerase enzyme (conc. 1 U μl^{-1}) and 16.8 µl of sterile PCR water (Genei, Bangalore)) and 2 µl (40–50 ng) of DNA sample. Amplification was carried out by 5 min of initial denaturation at 94°C followed by 40 cycles of denaturation of 94°C for 1 min; annealing at 37°C for 1 min; extension at 72°C for 2 min with a final extension at 72°C for 5 min.

PCR amplification and RFLP analysis of ITS region of rDNA

Amplifications were performed using universal primers ITS-1 and ITS-4 (White *et al.* 1990) in 0.2 ml Eppendorf tubes with 25 µl reaction mixture containing 2.5 µl of 10x Taq buffer, 2.5 µl of 25 mm $MgCl_2$, 2.0 µl of each primer (0.6 picomol μl^{-1}), 0.5 µl of 100 mM dNTP mix, 0.125 µl of *Taq* polymerase (0.5 U μl^{-1}) and 14.37 µl of sterile PCR water (Genei, Bangalore) and 3 µl (40–50 ng) of DNA sample. Amplification was carried out by 35 cycles, including denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1.5 min with initial denaturation at 94°C for 4 min before cycling and final extension at 72°C for 6 min. Amplified PCR products were digested with *Alu*I, *Hinf*I and *Mse*I restriction enzymes as per the manufacturers' instructions, and banding patterns were documented through a gel documentation system (Alpha Innotech).

Results and discussion

Survey, isolation and identification of pathogen

Maximum disease incidence (85%) was observed in Kattuluru village of Vempalli mandal in the Kadapa district, whereas the lowest disease incidence (1%) was observed in the Nallacheruvupalli village of Molakacheruvu mandal in the Chittoor district. The isolated pathogen was identified as *S. rolfsii* based on its mycelial and sclerotial characteristics through standard mycological keys. Isolates collected from Chittoor and Kadapa districts were designated as CSr 1 to CSr 10 and KSr 11 to KSr 20, respectively (Table 1).

Cultural and morphological variability

The isolates of *S. rolfsii* varied in all of the test parameters, viz. colony morphology, total growth, growth rate, time taken for sclerotial production, size, colour and number of sclerotia produced per plate (Table 1). Out of 20 isolates, colonies of 11 isolates showed fluffy growth, whereas 9 isolates were compact. However, all the isolates produced white cottony mycelial growth. Maximum radial growth was recorded for the isolates CSr 2, CSr 3, CSr 4, KSr 12, KSr 14, KSr 15, KSr 16, KSr 17 and KSr 18 (90 mm) and the least growth was observed for KSr 19 (62.7 mm). Maximum growth rate was observed for isolate KSr 15 (34.5 mm d^{-1}) and the least growth rate was observed for the KSr 19 isolate (15.6 mm d^{-1}). Isolates CSr 5, CSr 8 and KSr 11 took a maximum time of 18 days to produce sclerotia, whereas isolate CSr 4 took only 9 days. The largest sclerotia were produced by isolate KSr 16 (2.2 mm) and the smallest by isolate CSr 4 (0.90 mm). A maximum radial growth of 90 mm by an isolate *S. rolfsii* was also reported by Akram *et al.* (2007) for an isolate from chickpea and by Kulkarni *et al.* (2008) for an isolate from potato. Sarma *et al.* (2002) reported that the growth rate of different isolates of *S. rolfsii* varied from 23 to 31 mm d^{-1}. Okereke and Wokocha (2007) recorded a minimum sclerotial size of 0.8 mm and a maximum of 1.4 mm in a tomato isolate of *S. rolfsii*.

The results of the present study reveal wide variation among the *S. rolfsii* isolates in phenotypic characteristics, which could be due to differences in nutritional requirements and genetic characteristics. For example, osmotic and matric potential in the growth medium can influence the formation of reproductive structures by soilborne fungi (Cook and Duniway 1980). Other environmental factors that can influence sclerotial production include temperature, type and amount of nutrients and C:N ratio. In this study, variability studies were conducted by inoculating equal quanta of inoculum (5 mm colony discs) on a defined medium, so differences in genetic characteristics would have contributed to the differences observed.

Table 1. Percent disease incidence (PDI) of stem rot in the field and the cultural, morphological and *in vitro* pathogenic variability among the isolates of *Sclerotium rolfsii.* DAI = days after inoculation, RB = reddish brown, DB = dark brown and LB = light brown.

Isolate	Site	PDI in field (%)	Time taken for disease expression (DAI)	PDI in glasshouse (%)[1]	Total growth (mm)	Growth rate (mm/day)	Colony type	Time taken for sclerotia production (days)	Colour of sclerotia	No. of sclerotia (per plate)	Size of sclerotia (mm)
CSr1	Kottapalli	11.1	15	40 (39.2)	73	23	Fluffy	16	RB	81	1.42
CSr2	Kalikiri	6.7	8	53 (46.9)	90	30	Compact	13	RB	138	1.80
CSr3	Teegalakona	15.0	10	40 (39.2)	90	31	Compact	15	KB	347	0.92
CSr4	Taragonda	20.0	11	53 (46.9)	90	31	Compact	9	DB	385	0.90
CSr5	Yellampalli	2.0	10	47 (43.1)	68	18	Fluffy	18	LB	53	1.50
CSr6	Kothapalli	5.0	7	60 (50.8)	66	26	Fluffy	15	LB	30	1.50
CSr7	K. Kuruvapalli	30.0	16	40 (39.2)	73	23	Fluffy	15	DB	130	1.31
CSr8	Mudivedu	10.0	12	53 (45.9)	69	24	Fluffy	18	RB	58	2.00
CSr9	Nallacheruvu-palli	1.0	8	100 (90.0)	83	21	Fluffy	13	RB	185	1.63
CSr10	Peddapalem	3.0	8	27 (31.0)	83	23	Compact	11	DB	281	1.13
KSr11	Battalapalli	5.6	12	93 (31.1)	72	23	Fluffy	18	RB	64	0.95
KSr12	Gollapalli	5.8	10	87 (72.3)	90	33	Compact	16	RB	242	1.12
KSr13	Nagalagutta-varipalli	8.3	16	20 (26.6)	82	24	Fluffy	17	LB	257	1.48
KSr14	Kattuluru	85.0	8	60 (50.8)	90	33	Compact	15	LB	168	2.00
KSr15	Gangammapeta	5.7	12	20 (26.6)	90	34	Fluffy	13	RB	56	2.00
KSr16	Animela	30.0	15	27 (31.0)	90	29	Fluffy	14	RB	81	2.20
KSr17	V.N. Palli	13.3	15	53 (45.9)	90	30	Compact	14	DB	151	1.50
KSr18	Vuruturu	10.0	7	80 (63.4)	90	30	Compact	16	LB	571	2.00
KSr19	Sunkesula	10.0	10	100 (90.0)	62	15	Compact	12	LB	190	1.00
KSr20	Telagandlapalli	5.0	6	100 (90.0)	67	24	Fluffy	17	LB	122	1.62
Critical difference (P = 0.05)				(9.9)	0.7						
Standard error of mean				(±3.5)	±0.25						

[1]The figures in brackets are angular transformed values

Pathogenic variability

Table 1 shows that isolates CSr 9, KSr 19 and KSr 20 exhibited maximum disease incidence (100%) following inoculation on peanut, followed by KSr 11 with 93%. The lowest disease incidence was recorded for isolates KSr 13 and KSr 15 (20%). Dela-Cueva and Natural (1994) studied the pathological variation among the isolates of *S. rolfsii* from different crops. The cowpea isolate was considered the most aggressive and most virulent. Ansari and Agnihotri (2000) reported a positive correlation between oxalic acid production and the virulence of the *S. rolfsii* isolates. In this study we have not measured oxalic acid or other enzyme production by the selected isolates. Shukla and Pandey (2008) showed the pathogenic variability of ten *S. rolfsii* isolates recovered from diseased parts of *Parthenium*. Further they reported that isolate Par#02 showed the greatest disease incidence (80%) whereas isolate Par#10 showed least (30%). The spread of isolates in a particular geographic regions followed by changes in the genetic background through selection, adaptation or mutation could result in isolates with differing genetic composition and suggest that there might be several strains within this species. Such information on variability within the populations in a geographic region contributes to the growing knowledge of the biology and epidemiology of this economically important pathogen and assists the development of effective control strategies.

Genetic diversity among *S. rolfsii* isolates RAPD analysis

Random primers, viz. OPA-01, OPA-12, OPA-17, OPA-18 and OPA-20 generated a total of 221 reproducible and scorable polymorphic bands ranging approximately from as low as 100 bp to as high as 2500 bp among eight isolates (Fig. 1A-E). Figure 1F shows a dendrogram generated using the UPGMA package based on Ward's Squared Euclidean Distance method.

Based on the results obtained, all eight isolates were grouped into two main clusters. Cluster I contains four isolates, viz. CSr 9, KSr 17, CSr 1 and KSr 19 of which the first two and last two form two separate sub-clusters. Cluster II contains four isolates, viz. CSr 2, CSr 10, KSr 15 and KSr 20. The RAPD profiles show a distinct banding pattern indicating the presence of polymorphism among the isolates of *S. rolfsii.* Primer OPA-01 amplified two unique fragments of about 100 bp and 250 bp in case of isolate KSr 19. Primer OPA-12 amplified specific bands of 1400 bp and 1500 bp in the case of isolate KSr 15. Moreover, a 600 bp fragment was absent in the case of isolate CSr 1 compared to other isolates. OPA-17 primer yielded a specific fragment of about 200 bp in isolates CSr 9 and KSr 17 and was absent in all other isolates. Bands of 650 and 700 bp were specifically amplified in the case of isolate KSr 20, showing the polymorphism among the isolates. Primer OPA-18 amplified a unique band of about 500 bp in CSr 1, 575 bp in CSr 9 and 750 bp in case of KSr 20. Primer OPA-20 amplified a fragment of 1000 bp and 1050 bp in both KSr 15 and KSr 17 isolates. Results on RAPD profiles showed that the two highly virulent isolates grouped in one cluster (cluster I), suggesting similarities in genetic and/or virulence characteristics between isolates. However, additional and more detailed studies are required to confirm the relationship and its overall significance. Punja and Sun (2001) used RAPD analysis to study genetic diversity within and among mycelial compatibility groups of *S. rolfsii* and *Sclerotium delphinii*. The extent of genetic diversity among isolates of *S. delphinii* was lower than that observed in *S. rolfsii*. Almeida *et al.* (2001) studied variability among 30 isolates of *S. rolfsii* from different hosts in Brazil using RAPD profiles and identified 11 haplotypes. Saude *et al.* (2004) reported genetic variability among 17 isolates of *S. rolfsii* using mycelial compatibility groups (MCGs) and RAPD-PCR. RAPD analysis revealed similarities in banding patterns of isolates belonging to the same MCG with primer 335. RAPD profiles in this study clearly show distinct polymorphism among the isolates. The two unique fragments of about 100 bp and 250 bp in case of KSr 19 isolate with primer OPA-01 and 650 and 700 bp amplicons in case of KSr 20 were amplified with OPA-17 primer. The cloning and sequencing of these unique fragments from virulent isolates KSr 19 and KSr 20 could allow the design of specific oligonucleotides in order to develop SCAR markers for the detection of highly virulent isolates. Schilling *et al*. (1996) reported the development of SCAR markers based on RAPD profiles for *Fusarium* spp. Literature evidence for molecular genetic and virulence characteristics is variable and detailed characterisation of genetic differences (e.g. presence of a specific band or sequence) may be needed to identify links to virulence characteristics (Ramsay *et al.* 1996; Back *et al.* 1999).

For example, screening of isolates using a diverse set of RAPD primers belonging to different operon series or analysis using 'inter-simple sequence repeat – PCR' (ISSR-PCR) may help to find relationships, if any, between pathogenicity and taxonomic genetic characteristics.

ITS-PCR and ITS-RFLP analysis

Amplification of the ITS region of rDNA produced an approximately 650–700 bp fragment which is specific to *S. rolfsii* (Fig. 2 A). Our results are in agreement with those of Adandonon *et al.* (2005) who studied genetic variation among *S. rolfsii* isolates of cowpea from Benin and South Africa by using mycelial compatibility and ITS rDNA sequence data and obtained an amplification fragment of about 700 bp which is specific for *S. rolfsii*. In the present study, all isolates gave the same size of the fragment, that is 650–700 bp, which suggests that these isolates are the same species.

Harlton *et al.* (1995) screened a world-wide collection of *S. rolfsii*, using universal primer pairs ITS1-ITS4, ITS1-ITS2 and ITS3-ITS4, and revealed variation in ITS regions with 12 sub-groups. *Sclerotium rolfsii* and *S. delphinii* yielded a common unique band of about 720 bp.

ITS-RFLP results in this study showed similar restriction banding patterns among all the isolates for the restriction enzymes *Alu*I, *Hinf*I and *Mse*I (Fig. 2B–D). This shows that the restriction sites

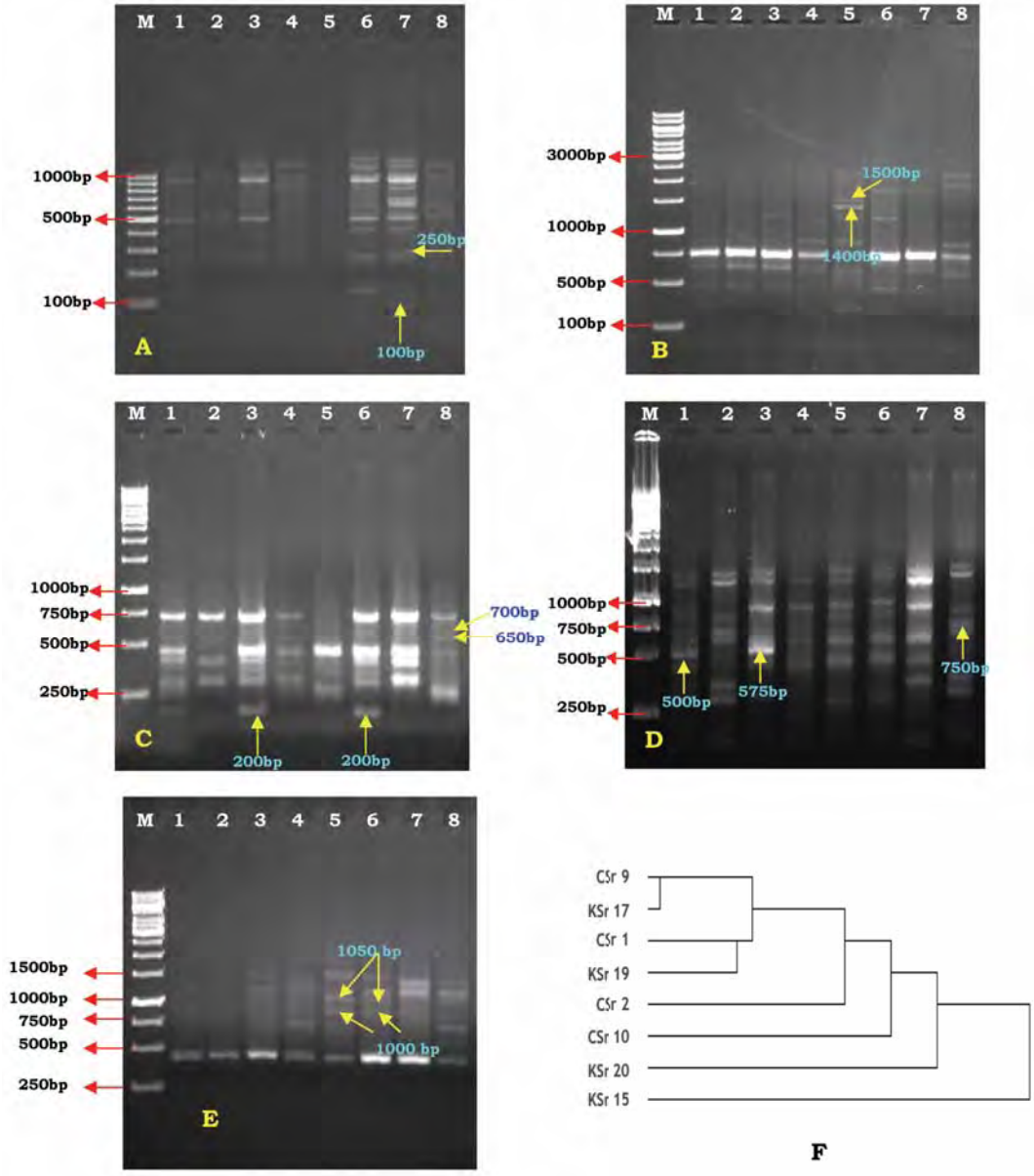

Figure 1. Genetic diversity of *S. rolfsii* isolates based on random amplified polymorphic DNA (RAPD) patterns generated using primers OPA-01 (A), OPA-12 (B), OPA-17 (C), OPA-18 (D) and OPA-20 (E). Lanes 1–8 represent *S. rolfsii* isolates CSr 1, CSr 2, CSr 9, CSr 10, KSr 15, KSr 17, KSr 19 and KSr 20, respectively. Lane M = 1kb DNA ladder. Figure 1F: Dendrogram generated using UPGMA analysis showing polymorphism among isolates of *S. rolfsii*.

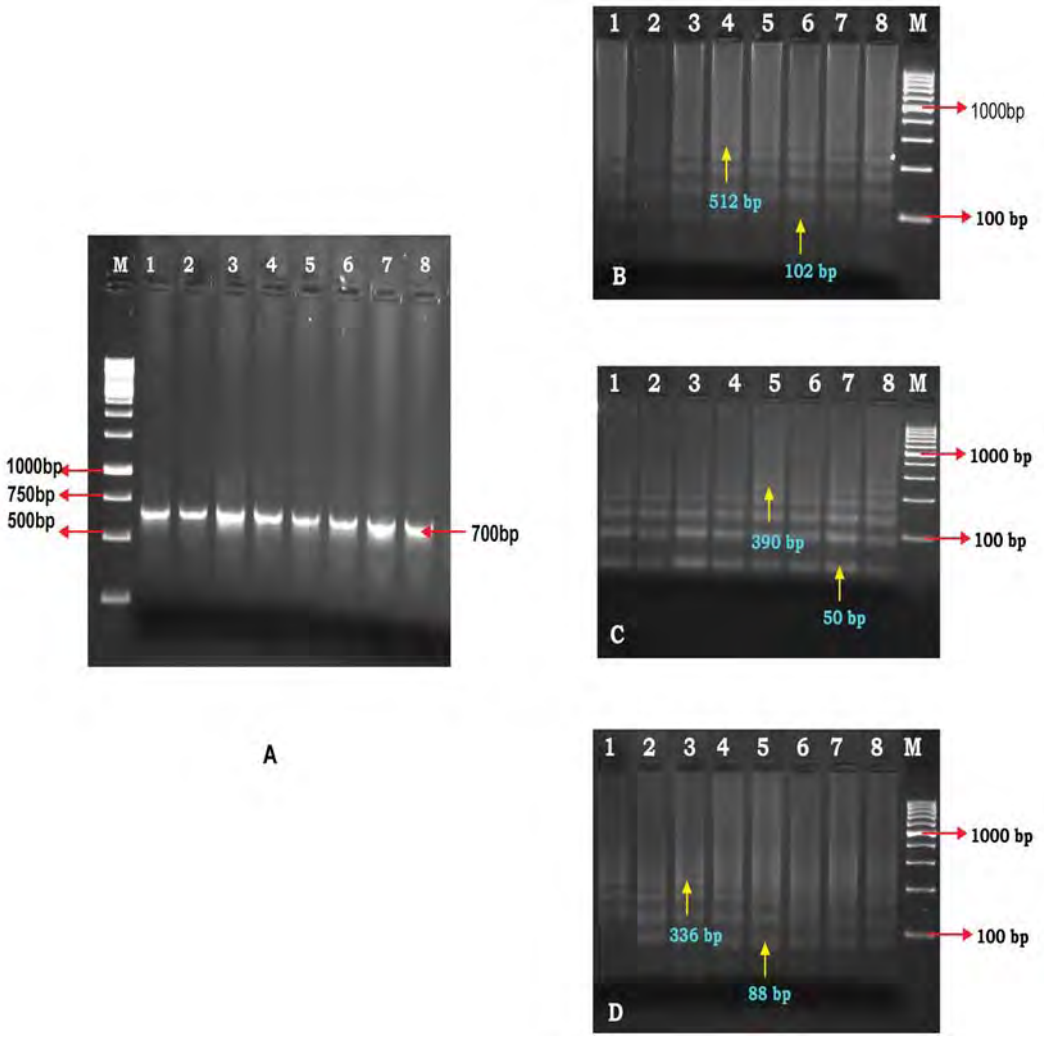

Figure 2. A: Amplification product of internal transcribed spacer (ITS). B–D: Restriction enzyme digestion of ITS-rDNA from different isolates of *S. rolfsii*: B: *Alu* I; C: *Hinf* I; D: *Mse* I. Lane M = 100 bp DNA ladder; lanes 1–8 are *S. rolfsii* isolates CSr 1, CSr 2, CSr 9, CSr 10, KSr 15, KSr 17, KSr19 and KSr 20 respectively.

for these enzymes were the same among all the isolates in the study, whereas Okabe *et al.* (1998) divided 67 isolates of the southern blight fungus (*Sclerotium* spp.) from Japan into five groups based on ITS-RFLP analysis of nuclear rDNA. Three groups were re-identified as *S. rolfsii* and two resembled *S. delphinii* in RFLP patterns. Almeida *et al*. (2000) classified 30 isolates of *S. rolfsii* from Brazil into two groups based on restriction analysis of PCR fragments. In the present study, the dendrogram developed using ITS sequences of *S. rolfsii* showed two main clusters, Cluster I and Cluster II. Cluster I contained the isolates CSr 1 CSr 2, CSr 9, CSr 10, KSr 15, KSr 17 and Cluster II containing the isolates KSr 19 and KSr 20. The authors are of the opinion that generating more information using RAPD profiles with different primers and the nucleotide sequences of ITS regions will lead to the further development of molecular diagnostics with respect to pathogenic variability and genetic diversity among *S. rolfsii* isolates. Molecular characterisation of fungal pathogens using RAPD marker

profiles and ISSR-PCR analysis has been effectively used previously for soilborne fungal pathogens, both to determine their geographic genetic variation and identify its relationship to pathogenicity (e.g. Back *et al.* 1999; Stodart *et al.* 2007).

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Development of a microbial indicator database for validating measures of sustainable forest soils

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Abstract

EdIRT (Edaphic Indicator Research Tool) is a database of microbial indicators of soil status, designed to assist in the validation of forest soil conservation efforts. The database prototype was developed using Microsoft Inc. (MS) Access, with regular migration to an open-source version using a structured query language (MySQL). To enable future web access, a browser-accessible interface was developed for the MySQL version of the database, using a mixture of personal home page language (PHP), cascading style sheet language (CSS), and hyper-text markup language (HTML). To enable queries for genetic sequences, a 'Pattern Recognition Algorithm for Microbes' (PRAM) was developed by modifying the Boyer-Moore algorithm. The database catalogs source information, genetic features, biochemical and physiological attributes, and functional significance of microbial indicators. EdIRT currently comprises a relatively modest dataset of 105 genetic sequences for microbial indicators of site productivity and tree loss in coastal Douglas-fir forests of British Columbia (BC). When 46 indicator sequences from EdIRT were used to re-evaluate source soils using molecular microarrays, 28 were actually ubiquitous, nine varied with season, four varied with site productivity, and three (a *Paenibacillus* sp., an unidentified member of the Intrasporangiaceae, and an unidentified diazotrophic bacterial species) were indicators of clear-cutting. Taxonomic analysis of sequences in EdIRT revealed some clustering of diazotrophic bacteria sequences related to the level of tree removal and season. Further expansion of EdIRT will allow for increased utility of the dataset in validating forest soil conservation efforts.

Introduction

Soil fertility is a prerequisite for sustainable forest ecosystems. One way to measure soil fertility is to measure the amount of available nutrients (N, P, K, etc.). This approach takes a 'snapshot' of soil status; it is a reasonable method for assessing general soil conditions at a point in time. Other indicators such as soil moisture and bulk density measurements can be helpful in determining the extent of disturbance impacts and some underlying mechanisms of disturbance. These approaches are inexpensive and provide immediate answers concerning soil conditions. However, they do not necessarily demonstrate sustainability, because they do not reveal underlying mechanisms controlling the dynamics of nutrient cycles, nor do they necessarily predict soil status in the long term. For example, forest sites with low available soil N might or might not have a problem with soil fertility, depending on the long-term rate of N-fixation and net N-loss due to denitrification. Likewise, sites with good nutrient availability at early stages of forest succession might not remain that way; if the diversity of key microbe commu-

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nities is reduced; their function at later stages of succession may be impaired or degraded. Thus, questions remain concerning the long-term impact of various types of forest disturbance, including forest management practices such as variable tree retention and clear-cutting.

Soil microbes (bacteria, fungi and protista) are much more prevalent than other potential indicators, in some cases approaching 10 000 different species per gram in forest soil (Chatzinotas 1998; Quince et al. 2008). Microbes are a very attractive measure of soil health because they are the drivers of about 80–90% of all soil nutrient processes (Quince et al. 2008). Soil microorganisms are challenging to monitor; only 5–10% of soil bacteria are culturable according to some estimates (Janssen et al. 2002). However, molecular profiling methods can be used to catalogue and understand the taxonomic and functional diversity of soil microorganisms, which can help elucidate the functional behavior of these communities. There is currently a lack of data regarding links between microbiological diversity and ecosystem function. Changes to microbiological diversity can be linked to alterations in ecosystem process rate changes (Allison and Martiny 2008), but the high levels of microbial diversity and abundance in soil ecosystems can buffer the impacts of disturbance on functional attributes of communities. On the other hand, ecological succession or disturbance in soil ecosystems may open or close microbial niches, allowing specific groups of microorganisms to increase or decrease in abundance. The ability to detect and catalogue the changes to microbial community structure may aid in the identification of specific DNA sequences linked to dynamic microbial populations. Large-scale fluctuations in these dynamic microbes potentially have important impacts on soil fertility, but they cannot be fully understood until the key species are detected and catalogued for further evaluation in quantitative studies. Databases are tools that can be used to gather indicator information to one common point of reference, to increase the power of analyses and to improve the long-term prospects for practical extension of results (e.g., validation of soil indicator methodologies). Through amassing microbial DNA sequence data and associated information concerning environmental conditions, researchers can potentially filter these data, using selected environmental criteria to uncover trends of functional significance. Moreover, databases facilitate the comparison of indicator suites or profiles in a complex, diverse environment where the occurrence of individual indicators may be highly variable. All of these things would benefit researchers and forest managers seeking to understand the behavior of these indicators in edaphic environments. We developed and tested a database to expedite the use of microbial indicators in validation of soil health measurements for sustainable forest management.

In British Columbia, Canada (BC), forest soil conservation guidelines are detailed in the *Forest Resource Protection Act* (FRPA). The long-term objective of the database development outlined in this paper is to support these FRPA guidelines by providing a means to evaluate their effectiveness, using microbial indicators of common soil disturbances and key nutrient-cycling functions. The specific short-term objectives of this project were to:

- develop a microbial indicator database called EdIRT (the Edaphic Indicator Research Tool) using Microsoft© (MS) Access™ (Microsoft Corp., Redmond, WA, USA)
- develop a personal home-page language (PHP)-based interface for an open-source version of EdIRT permitting sequence queries and Internet browser access
- test the power of indicators in EdIRT using taxonomic analyses and a DNA microarray assay.

Materials and methods

Database development

EdIRT was developed as a relational database to catalog source information, genetic sequences, biochemical attributes, physiological attributes and functional information associated with microbial indicator signals (Fig. 1). EdIRT was initially developed using commercial software (MS Access™). This software allowed the development of custom data entry forms and simple custom queries that could be associated with the data tables. It was necessary, however, to develop an alternate version to facilitate eventual Internet access and more advanced queries relating to DNA sequences. Commercial software (DBManager 3.1.0, DBTools Inc., Brazil) was used to migrate data from Access tables to a MySQL 5.0 library of tables accessible through an Apache 2.0 hyper-text markup language

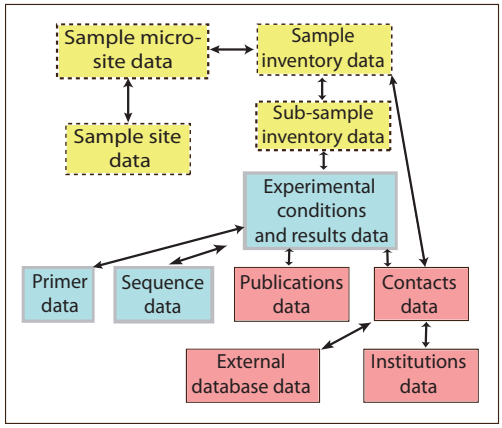

Figure 1. An overview of the principal relationships in the EdIRT relational database

(HTML) server through a PHP-based interface. The web-based interface was written using a combination of HTML, PHP and cascading style sheet (CSS) languages. The HTML provides the basic structure of each page, while CSS provides a common design that is easily updated independently of each separate page. The PHP language allows server-side functions by interacting with the MySQL database program. This scripting language allows the dynamic queries of a web-user to interact with data in a separate database. The primary web-based interface file, edirt.php, is able to call all other PHP interface files. The edirt.php file also coordinates an EdIRT function called the overall pattern recognition algorithm for microbes (PRAM). When the PRAM is activated, edirt.php calls sequencesearch.php, which in turn calls the file functions.php and its pattern recognition algorithms. With some modifications, PRAM functions are based on the Boyer-Moore algorithm that is also used by the US National Center for Biotechnology Information discontinuous megaBLAST program[1]. PRAM compares base pairs of known and unknown sequences, starting with the 5' end and skipping non-matching base-pairs. The process is repeated with other sequences, and a score based on sequence similarity is obtained. Sequences are displayed in order of their ranked scores; queries linked to other data tables become available for each specified sequence.

[1] Available online at: http://www.ncbi.nlm.nih.gov/blast/megablast.shtml

Collection, extraction and determination of indicator sequences

Molecular sequences included in EdIRT were sampled at field sites located on Vancouver Island, BC, Canada. Soil samples were collected in the Shawnigan Lake (lower productivity) and Sayward Forest (higher productivity) Level of Growth Stock (LOGS) installations with 63- and 61-year-old second-growth Douglas-fir plantations, respectively. Sampling took place in 0.5-ha second-growth forest plots including organic and mineral soils from control, highly (90%) thinned (35-year impact), and recently (< 3 year) clear-cut plots. Because tree roots and mycorrhizae often occupy the totality of bulk samples, these were all effectively considered to include rhizospheric species. Ten samples from each plot were pooled. The MoBio UltraClean™ Microbial DNA Isolation Kit (MoBio© Laboratories Inc., Carlsbad, CA, USA) was used to extract DNA from the soil samples, modifying the manufacturer's protocol to remove humic substances. 260 µl of 1x Tris-EDTA (TE) (pH 8) buffer was added to the post-extracted DNA with 40 µl ammonium acetate buffer and 6 µl of linear polyacrylamide co-precipitant to aid alcohol precipitation of DNA (Bioline Ltd, London, UK). The mixture was incubated at 20°C for 15 min and centrifuged at 12 500 rpm. The DNA pellet was isolated and washed with 70% EtOH, followed by a second centrifuge step. The pellet was air-dried and suspended in 50 µl TE. DNA was stored at –20°C prior to PCR. Polymerase chain reaction (PCR) amplification and denaturing gradient gel electrophoresis (DGGE) analysis were performed using oligonucleotide primer sets described in Tables 1 and 2.

DGGE was performed using the BioRad© (Hercules, CA, USA) Universal Mutation Detection System™ with at 6% (w/v) acrylamide / bisacrylaminde stacking gel with no denaturant and a separating gel of 8% polyacrylamide with a denaturing gradient (urea and formamide) of 35–65%. Gels were stained for 20 min in 1x TAE with SYBR Gold and documented using a transillumination system (Syngene, Cambridge, UK). Dominant and unique bands were excised from the acrylamide gel. Excised bands were deposited into 160 µL sterile dH_2O and a freeze-thaw cycle was used to physically disrupt the gel as described in Bäckman *et al.* (2004). DNA was re-extracted from the disrupted gel and sequenced by Macrogen Inc. (Seoul, Korea). Post-DGGE DNA extracts were sequenced and added to the EdIRT database. Currently, thirty

Table 1. Primer sets and functional targets used for PCR-DGGE analysis of soil microbial communities

Primers	Functional target(s)	Reference
16S – Actinomycete specific	Multiple functions (e.g. N-fixation)	Heurer *et al.* 1997
16S – *Paenibacillus*-specific	Multiple functions (e.g. N-fixation)	da Silva *et al.* 2003
16S – N oxidiser specific	Nitrification	
16S – CTO β-subgroup	Ammonia oxidisers	
amoA	Autotroph. ammonia-oxidisers	Stephen *et al.* 1999
nifH – nitrogenase (universal, *Azotobacter*)	Nitrogen fixation	Bürgmann *et al.* 2004
nirK – nitrite reductase	Denitrification	Chénier *et al.* 2004
18S – fungal specific	Multiple functions, e.g. mycorrhizae	
ITS1F	Ectomycorrhizal basidiomycetes	Gardes and Bruns 1993
NLB4	Ectomycorrhizal basidiomycetes	Smit *et al.* 2003

nifH DNA sequences are also deposited in a public database (GenBank accession numbers EU730607 – EU730636).

Taxonomic analyses

Taxonomic analysis was performed with *nifH* sequences obtained from DGGE following their deposition into the EdIRT database. The sequenced *nifH* gene fragments were aligned with published *nifH* sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/) using the CLUSTAL W 2.0.6 program (Thompson *et al.* 1994). Phylogenetic analysis was performed using the neighbour-joining method on a 323 bp *nifH* fragment that excluded both primer sites to allow phylogenetic analysis with a maximum number of published *nifH* reference sequences using BioEdit Sequence Alignment Editor 7.0 (Hall 1999) and the TreeView 1.6.6 plug-in using 100 bootstrap replicates.

DNA microarray analysis

A DNA (35-mer) microarray was constructed from sequences in EdIRT (Table 2). From excised DGGE bands, 19 Eubacterial, 19 *Paenibacillus* sp., 15 Acintomyces and 28 *nifH* bands were chosen as candidates for use in the microarray. Sequences were compared for areas of divergence from homologous sequences. Contiguous 35-nucleotide sequences were located in divergent areas to obtain sequence-specific oligomers. The oligomers were checked for cross-reactivity, hairpin formation and melting temperature. Any potential oligomers that formed secondary structures or were not within acceptable thermodynamic parameters were not used. The 46 sequences (35-mer) making it to the final stage (e.g., Table 2) were synthesised by Integrated DNA Technologies (Coralville, IA, U.S.A.), and spotted on microarray slides at the National Research Council Biotechnology Research Institute in Montréal. Cy-5 labeled DNA extracted from positive controls (*Bacillus* sp., *Frankia* sp.), negative controls (*Alcinovorax* sp.), and new soil samples was placed on the slides, hybridised over-night, and removed by washing. The soil samples corresponded to the original isolation sites for the indicators. Fluorescent spots indicating matching oligomers were detected with a laser microarray scanner.

Results and discussion

EdIRT now contains 105 DGGE-detected DNA sequences corresponding to microbial indicators of forest disturbance in coastal Douglas-fir forests of the Pacific Northwest. It currently comprises 29 main data tables (Table 3), six sub-tables, one linking table and one large soil sub-group table applicable to Canadian soil classes.

Taxonomic analysis of sequences in EdIRT revealed that some sequences corresponding to diazotrophic bacteria clustered according to the level of tree removal and season (Fig. 2).

In the DNA microarray assays, no negative controls fluoresced and all positive controls successfully hybridised with their intended targets, although cross-reactivity between a labeled *Frankia* spp. *nifH*-oligomers and 16S DNA from an uncultured *Acinetobacterium* spp. clone was noted. In the assay, 28 sequences were ubiquitous, nine varied with

Table 2. Some of the bacterial indicators from EdIRT included in the microarray assay. Sequence identities correspond to the most like sequence suggested by the NCBI discontinuous MegaBLAST program.

Organism	PCR primer set	Forward primer(s) (5′-3′)	Reverse primer(s) (5′-3′)
Azotobacter vinelandii DSM576	16S 341F/758R[1]	CCTACGGGAGGCAGCAG	CTACCAGGGTATCTAATCC
Bacillus chitinolyticus	16S 341F/907R		
B. mucilaginosus strain KNP414	16S 341F/907R		
Frankia sp.	16S 341F/907R		
Rhodococcus polyvorum	16S 341F/907R		
R. rhodocorus	16S 341F/907R		
Stenotrophomonas sp. FL-2	16S 341F/907R		
Luedmannella helvata	16S F234/R1378 and F234/R513[2]	GGATGAGCCCGCGGCCTA and AACGCGAAGAACCTTAC	CGGCCGCGGCTGCTGGCACGTA and CGGTGTGTACAAGGCCCGGGAACG
Paenibacillus hodogayensis	16S 515F/R1401 and F968-GC/R1401[3]	GCTCGGAGAGTGACGGTACCTGAGA and AACGCGAAGAACCTTAC	GCGTGTGTACAAGACCC
P. mendelii	16S[3] 515F/R1401 and F968-GC/R1401		
P. polymyxa (2 strains)	16S[3] 515F/R1401 and F968-GC/R1401		
Azorhizobium caulinodans	*nifH* Fa/R and Fb/R[4]	GCIWTITAYGGNAARGGNG and GGITGTGAYCCNAAVGCNGA	GCRTAIABNGCCATCATYTC
Azospirillum brasilense	*nifH* Fa/R and Fb/R		
Bradyrhizobium japonicum	*nifH* Fa/R and Fb/R		
Burkolderia cepacia	*nifH* Fa/R and Fb/R		
Methylocystis sp. LW2	*nifH* Fa/R and Fb/R		
Methylobacterium nodulans	*nifH* Fa/R and Fb/R		

[1] Fortin *et al.* (2004) and Muyzer *et al.* (1993); [2] Heuer *et al.* (1997); [3] da Silva *et al.* (2003); [4] Bürgmann *et al.* (2004)

Table 3. Data included in the EdIRT database

Name of data table	Description	Fields
CONTACTS	Data contributors	7
INSTITUTIONS	Contributing institutions, group or lab	10
PUBLICATIONS	Publications associated with the entered data	6
AUTHOR	Authors of published articles above	3
CULTURE	Culture method and composition	6
PRIMER	Primers used for microbial identification methods	8
SEQUENCES	DNA sequences	9
TAXONOMY	Proposed taxonomy of indicator	13
results_BIOLOG	BioLog assay and carbon source results	15
results_ENZYME	Enzyme assay results	14
results_MCRARRAY	Microarray assay results	13
results_MCRB_BIOMASS	Microbial biomass results (carbon measurement)	14
results_PCR	Results of PCR-based assays (qPCR, DGGE, T-RFLP, etc.)	20
results_PLFA	Results of Phospholipid Fatty-acid Analysis	14
results_SIR	Results of Substrate Induced Respiration assays or catabolic profiling plate assays	17
results_GENERIC	Generic results for assays not included above	12
SAMP_INVENTORY	Sample description, collection, transport, storage	16
SUBSAMP_INVENTORY	Sub-sample description	12
SAMP_SITE	Physical, chemical, ecological and climatic attributes of the sample location	10
SAMPSITE_DESCRIP	Detailed description of a sample site	14
LRG_TREE_COMP	Ten most frequent tree species at the sample site	8
UNDERFSTOREY_COMP	Understory bushes, shrubs, herbs, etc.	8
SITE_ORIGIN	Origin of vegetation on the sample site	6
SITE_TREAT	Treatments applied to the sample site	6
SITE_DISTURB	Disturbances experienced at the sample site	6
SAMP_MICROSITE	Micro-sites sampled within the larger sample site	19
CONTAMINANTS	Description of contaminants at sample site	5
FUNGI_COMP	Details of associated fungal species at sample site	7
BACT_COMP	Details of associated bacterial species at sample site	7

season, four varied with site productivity, and three (a *Paenibacillus* sp., an unidentified member of the Intrasporangiaceae, and an unidentified diazotrophic bacterial species) were consistent indicators of clear-cutting at specific sites. The EdIRT database was used to assemble sequence profiles according to associated edaphic factors, supporting the development of molecular tools designed to detect potential indicators of environmental disturbance, for example clear-cut logging. While the use of a database may not be required for selecting a small number of sequences to use in a small microarray, EdIRT has the potential to facilitate the analysis and validation of edaphic microbial indicators in much larger datasets and arrays. Given the vast number of forest ecosystems, ecological niches and disturbance types to be addressed in microbial indicator studies, databases like EdIRT may become increasingly necessary as gains in the quantity, scope and complexity of the data are realised. Implementation of a public (online) version of EdIRT is pending several refinements, including French translation modules and adjustment of the input–output architecture of the PHP interface. Further expansion of EdIRT will allow for increased utility of the dataset in evaluating forest soil conservation efforts in BC, Canada, and throughout the world's forests.

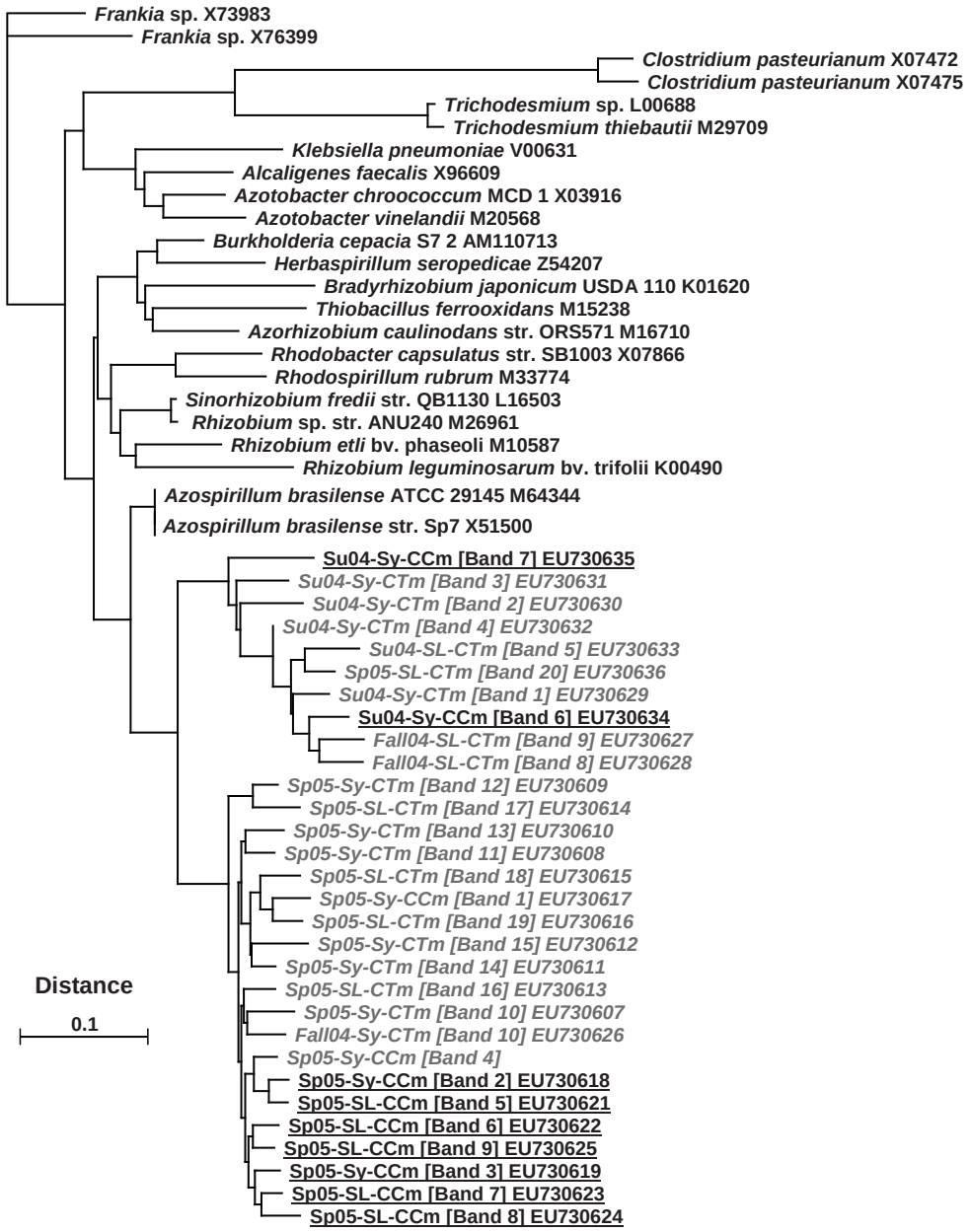

Figure 2. Cluster analysis of of sequenced nitrogenase reductase (*nifH*) from diazotrophic bacteria currently in EdIRT. The *nifH* gene fragments (position: 131-334 bp from start of *Azotobacter vinelandii* (M20568) *nifH* sequence) from Douglas-fir forest plots compared to known *nifH* sequences obtained from GenBank. Sequences were obtained following direct amplification from isolated DGGE bands from mineral layer soil DNA extractions from summer 2004(Su04), fall 2004 (Fall04) and spring 2005 (Sp05) sampling dates at Shawningan Lake (SL) or Sayward Forest (Sy) sites in clearcut (CC; underlined) or unthinned control (CT; grey italics) plots. GenBank accession numbers are listed beside their respective *nifH* sequence. *Frankia* sp. (X73983, X76399) were used for the out-group.

Acknowledgments

Funding for EdIRT development came from the CFS Enhanced Pest Management Methods Program and the British Columbia Forest Science Program. Portions of EdIRT were developed with the aid of CLPP profiles provided by Dr Sue Grayston (UBC). We thank Sandra Eades, Ryan Miller, Alex Gauld, Eric Bol and Shannon Berch for technical assistance.

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Long-term international research cooperation between China and Australia on soil biology in agriculture

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Abstract

Research cooperation between CSIRO Division of Soils and China Agricultural University (CAU, formerly Beijing Agricultural University) began following a visit by Dr Albert Rovira to China in 1993. Having identified a common interest in soil-borne constraints to crop production and the potential benefits to crop production of exploiting plant-associated microbes as inoculants, collaborative research projects have focused on 'yield-increasing bacteria' (YIB), and biological control agents for soil-borne diseases of crops. Initially a one-year pilot project (1994–1995) was supported by the Australian Centre for International Agricultural Research (ACIAR). Following the success of this project, ACIAR supported a larger, longer cooperative research program (1997–2001) on biological control of soil-borne root diseases. The research topics of the expanded cooperation included (i) the isolation and field testing of biocontrol agents on wheat, cotton and vegetables, (ii) investigation of mechanisms in growth and yield promotion, (iii) the microbial ecology of biocontrol agents and (iv) the ecology of soil-borne plant pathogens. Some commercial development of microbial treatments in China came from the collaborative research effort.

Subsequent bilateral R&D has been supported by grants from AusIndustry and the Ministry of Science and Technology (MOST) in China (2002–2004), followed by a grant from the Department for Education Science and Training (DEST) Australia – MOST China (2005–2007) and a grant from the Grains R&D Corporation (GRDC). Collaboration continues through a current Department of Innovation, Industry, Science and Research (DIISR) (Australia) – MOST (China) grant to CSIRO and three institutes in China to work on molecular mechanisms in biocontrol (2008–2010).

The research cooperation has led to the publication of scientific papers and has featured numerous exchange visits, conferences and training workshops, supported by ACIAR, the Crawford Fund and Chinese Government support for training. In 2005, members of the collaboration team played a critical role in the organisation and delivery of a Crawford Fund Master Class on soil-borne diseases of wheat in China. Subsequently there has been a broadening of the Australia–China collaboration to include the impact and control of cereal cyst nematode in China. The long-term collaboration has been beneficial to both China and Australia and has the potential to make significant further contributions to improving crop health and yield in both countries.

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Introduction

Australia and China share a strong interest in improving the efficiency of crop production to improve the effectiveness of land and resource use. One aspect of improved efficiency is increasing water-use efficiency by crops. Many factors determine water-use efficiency by a crop, including root growth and root health. In situations where there is severe root disease or where there are other soil-borne constraints to root growth, water-use efficiency can be dramatically reduced.

Optimum root health and root growth, to support good yields, can be best achieved by a combination of good soil structure (soil physics), good nutrition (soil chemistry) and an active soil food web and lack of pathogens (soil biology).

Since the 1980s, Chinese scientists had been researching and using 'Yield-increasing bacteria' or 'YIB' (Mei *et al.* 1990; Mei 1991). These YIB were plant-associated isolates of *Bacillus,* mainly *B. cereus.* The YIB were applied to many crops in the field and millions of hectares of crop were treated: for example there are reports of 40 million ha treated by 1994 (see papers in Tang *et al.* 1996). Average yield responses reported for the use of YIB ranged from 11% with wheat to 25% with vegetables (Mei *et al.* 1990; Mei 1991).

During a lecture tour of China by Dr Albert Rovira in 1993, a common interest in plant growth-promoting soil microbes was identified. Dr Rovira (at that time the director of the Cooperative Research Centre for Soil and Land Management) and Professor Tang Wenhua of Beijing Agricultural University mooted the concept of establishing a joint research project. A cooperative research project was established the following year, with funding from the Australian Centre for International Agricultural Research.

Research and development cooperation

Research cooperation Phase 1

The aims of the initial research project were to exchange beneficial soil bacteria and fungi between the CSIRO Division of Soils in Australia and Beijing Agricultural University (now China Agricultural University) in China and to test the ability of these microbial treatments to control economically important crop root diseases such as take-all (China and Australia) and sharp eyespot of wheat (China only).

At first, isolates of *Trichoderma*, *Bacillus* and *Pseudomonas* were tested in glasshouse experiments and then some of these 'biocontrol microbes' were formulated for seed application and tested in field trials in China. The results of these early tests were quite promising, with good levels of disease control and plant growth promotion (see papers in Tang *et al.* 1996).

The Chinese YIB strains proved to be very effective at promoting plant growth in glasshouse experiments in Australia (Ryder *et al.* 1999), but field tests were not so successful. This is likely to be due to the very different conditions encountered in the field in Australia (mainly dryland or rain-fed cereal production) compared to those in China, where there is much irrigated cereal production.

On the basis of success in the pilot project, a larger cooperative project (Phase 2) was proposed by the project leaders, Professor Tang and Dr Ryder.

Research cooperation Phase 2

Between 1997 and 2001 a larger ACIAR-funded project was established (ACIAR project LWR2/1996/080). In this cooperative project, the scope and number of partner organisations was broadened. The partners were China Agricultural University (CAU), Chinese Academy of Agricultural Sciences (CAAS) and Zhejiang Agricultural University (ZAU, now Zhejiang University) in China and CSIRO Division of Soils (later CSIRO Land and Water) and the Australian Cotton Research Institute (ACRI) in Australia. The teams were led by Professor Tang Wenhua (CAU), Dr Peng Yufa (CAAS), Professor Zhang Bingxin (ZAU), Dr Subbu Putcha (ACRI) and Dr Maarten Ryder (CSIRO).

The research aims of the larger ACIAR project were to:

- test the effectiveness and reliability of promising biological control agents for take-all and *Rhizoctonia* diseases of wheat under field conditions

- evaluate biological agents for reliable control of damping-off on solanaceous and cucurbitaceous vegetables
- evaluate the potential for biological control of damping-off and *Verticillium* wilt of cotton
- investigate the biological basis for biocontrol of soil-borne diseases and plant growth promotion.

The overall aim was to reduce the need for chemical fungicides or for cultivation treatments that could lead to soil erosion by developing effective and reliable microbial treatments that farmers could use to control soil-borne root diseases of the target crops.

Outputs and some of the main findings

Biocontrol of take-all and rhizoctonia on wheat

Work towards commercialising biocontrol in China culminated in the pre-registration of a formulated product of CSIRO's *Trichoderma koningii* (Tk 7a) by the CAU team. A licence agreement between CAU and CSIRO was signed at one of the sessions of the final review meeting in Beijing in 1999. One of the benefits of the bilateral cooperation was the input of Chinese expertise in the formulation of biocontrol micro-organisms into products that could be used by farmers. A commercial product based on the beneficial fungus *Trichoderma* Tk 7a was produced and sold in China by the MinFeng company (Shandong province) in 1999 and 2000. In addition, the Tk7a organism that had been formulated by the Chinese methods was used in experimental trials in Australia between 1999 and 2002.

Scientists at CAU developed a new bacterial biocontrol treatment that came from a disease-suppressive soil in Shandong province. The particular strain of *Pseudomonas*, CPF 10, was patented and registered in China for the control of bacterial wilt of ginger. Another soil bacterium, *Pseudomonas* Pf P303, which was isolated by CAAS scientists, gave good yield responses (11–23%) in large-scale field trials (2100 ha) in China, when used individually and in combination with the chemical fungicide Bayleton®.

A new selective medium was developed for *Trichoderma* spp. to allow isolation of the fungus from soils where it exists in low levels. New potential biocontrol agents (*Trichoderma* and *Bacillus*) were isolated from a disease-suppressive soil in South Australia. Finally, protoplast fusion of *Trichoderma* spp. produced progeny that had improved biocontrol ability. This research was done by Dr Yang Hetong, SDAS, while visiting CSIRO.

Biocontrol of damping-off diseases of solanaceous and cucurbitaceous vegetables

A number of new bacterial isolates were identified that had good ability to control damping-off diseases of vegetables. Microbial ecology techniques were taught by CSIRO scientists and were used extensively in this part of the research program at ZAU, led by Professor Zhang Bingxin. This included tracking of biocontrol agents in the soil and on plant roots using marker genes and an investigation of the ecology of the damping-off pathogen *Pythium*.

Biocontrol of damping-off and verticillium wilt of cotton

Excellent control of cotton seedling disease by binucleate (non-pathogenic) *Rhizoctonia* was discovered.

Biological basis for disease control and or growth promotion

Both the CAU and CSIRO groups demonstrated a role for antibiotics produced by selected CAU strains of *Bacillus subtilis* in their capacity to control root disease, and that this mechanism is independent of their plant growth promoting ability (this was only the second demonstration of this concept for *Bacillus*, worldwide).

Identification of common root rot problem in dryland wheat production

Cooperation between CAU and CSIRO scientists in 1998 led to the identification of a widespread and serious problem with common root rot (a fungal disease) in Shanxi province in central western China. This soilborne disease constraint had not previously been identified.

A report on this Phase 2 project, covering the application of the research as well as training and exchange visits, was included in an ACIAR Project Adoption Study (Ryder 2005). The research carried out under the first two cooperation projects from 1994 to 2001 generated 39 scientific papers, which are listed at http://www.clw.csiro.au/publications/consultancy/2003/ACIAR_report_App1only.pdf.

Research cooperation Phase 3

Following the research projects funded by ACIAR, several of the collaborating institutes sought and found funding to continue the collaboration. The first of these was a bilateral project funded by AusIndustry and the Ministry of Science and Technology (MOST) in China (AusIndustry project CH020003). This two-year cooperation (2002–2004) was aimed at connecting research and industry in both countries, to facilitate the commercial development of biological control agents.

The title of the project was 'Commercial application of disease control biotechnology to increase crop productivity' and the main partner institutes were CAU and CSIRO, with industry collaborators the Qinhuangdao Leading Science and Technology Company in China and Bio-Care Technology Pty Ltd in Australia (later Becker Underwood Australia Pty Ltd).

The partner companies received, from the research institutes, microbial cultures for formulation development and testing, as well as extensive sets of data on performance of microbial cultures in controlling disease. Through this process, some biocontrol agents such as *Trichoderma* Tk7a were found to have a broader spectrum of disease control activity than was previously recognised. The project fostered active links between science and industry, but it was recognised that the decision to develop a microbial treatment into a saleable product ultimately lies with the commercial organisation.

A second bilateral project (2005–2007), entitled 'New microbial inoculants and monitoring tools for agriculture via the formal establishment of joint research and development facilities', was jointly funded by the Department for Education Science and Training (DEST project CH040109) in Australia and MOST in China. Under this project, a formal cooperation agreement between CSIRO and SDAS was signed in September 2005. This coincided with an article about our long-term cooperation in a booklet that celebrated 30 years of CSIRO's involvement in China (CSIRO 2005).

Also under the auspices of this project, Chinese soil samples were analysed in Australia for plant pathogens using the South Australian Research and Development Institute (SARDI)–CSIRO DNA-based Root Disease Testing Service (available commercially in Australia as 'Predicta-B'). This initial set of tests gave promising results, which have since been followed up with testing of Chinese soils for nematodes that attack the roots of cereal crops.

Research cooperation Phase 4

After 2002, research cooperation on soil biology in agriculture expanded to include more scientists and institutes in both China and Australia.

From 2003 to 2006, the Grains R&D Corporation in Australia supported a collaborative project involving SARDI (Dr Steve Barnett) and the SDAS (Dr Yang Hetong) on the topic 'TRINOC: Trinoculation for enhanced growth of wheat under disease-limiting conditions'. Under this project, a visiting scientist from SDAS (Guo Yong) spent a year at SARDI working on the formulation of a mixture of organisms for the control of rhizoctonia bare patch disease.

Currently (2008–2010), the international collaboration continues through a project entitled 'Molecular mechanisms involved in biological control of root diseases by novel microbial inoculants'. This is a joint project involving CSIRO Entomology (Dr Paul Harvey) and Hebei Academy of Agricultural and Forest Sciences (Dr Ma Ping) as well as the SDAS (Dr Yang Hetong) and CAU (Dr Zhang Liqun). The project is financially supported by the Department of Innovation, Industry, Science and Research (DIISR, Australia) and MOST in China under the Australia–China Special Fund for Scientific and Technological Cooperation. The project involves workshops, exchange of technical information and active research cooperation at the laboratory and field level.

After the Crawford Master Class on soil-borne diseases of wheat (China 2005), collaboration between SARDI (Dr Ian Riley, Dr Kathy Ophel-Keller and others) and Chinese institutes (CAAS, CAU and others) was established on evaluating the significance of and ways to control cereal cyst nematode in China. This collaboration also includes the International Maize and Wheat Improvement Center (CIMMYT) (Dr Julie Nicol).

Workshops and symposia

During the course of the various collaborative projects since 1994, members of the cooperating teams have organised a number of conferences, symposia

and workshops. These gatherings have been significant in bringing people together, allowing exchange of information and fostering progress in the joint scientific research.

In 1996, an important international conference on biological control of plant disease was held in Beijing. The conference was instigated by Professor Tang Wenhua and the committee also included Dr Albert Rovira and Dr R. James Cook (USDA, USA) as leaders. About half of the conference delegates were from China and half from other countries. The proceedings of this conference were published by CAU press (Tang *et al.* 1996).

The final review meeting for project LWR2/1996/080 in Beijing in November 1999 consisted of two days of research presentations of a very high standard. The project was evaluated by two independent reviewers at this workshop and teams were given a substantial amount of positive feedback for their achievements.

In November 2002, under the AusIndustry–MOST project, a two-day workshop was held in Inner Mongolia on the biological control of root diseases. Scientists from CAU, CSIRO and other partner institutes in China were the presenters and over 50 local provincial scientists and government staff attended. As part of the same bilateral project, a symposium on 'R & D on new microbial inoculants for improved crop productivity in China and Australia' was held at CSIRO in Adelaide in May 2004. The speakers at this symposium were from CSIRO, SARDI, Shanghai Academy of Agricultural Sciences, Hebei Academy of Agricultural and Forest Sciences and Henan Agricultural University.

To celebrate ten years of research cooperation, a special symposium was held in November 2004 at the Biology Research Institute, Jinan, Shandong province. There were six speakers from Australia and eight from China. The collected papers from this symposium were published in a special issue of *Shandong Science* in 2005 (see Ryder *et al.* 2005).

Exchange visits and the training of scientists and students

One of the achievements of the long-term collaboration is the large amount of time that has been devoted to training and exchange visits. This is also one of the reasons why the cooperation has continued successfully for such a long period.

For example, during phase 2 (the larger ACIAR-funded project), three Chinese scientists (Yang Hetong, Tu Haidy and Lou Binggan) each spent 9 –12 months working at CSIRO in Adelaide. Albert Rovira and Maarten Ryder each spent three months in China (in 1997 and 1998 respectively), where they worked closely in the laboratory with the Chinese research teams and gave lecture series. They worked mainly at ZAU in Hangzhou, but also in Beijing and Shandong Province. At Hangzhou, one of the main tasks was to assist with re-starting a biocontrol research program. Stephen Neate and Paul Harvey each spent several weeks in China in 1998 and 1999 respectively, to visit field sites and to give lectures.

Another example is the more recent collaboration between SARDI and SDAS in which a Chinese scientist spent over nine months working at SARDI in Adelaide. These longer-term visits have enabled strong relationships to be built, and have led to a great deal of training and exchange of information, expertise and technology.

The Crawford Fund for International Agricultural Research supported two medium-term visits in the earlier stages of the collaboration: Professor Peng Yufa visited CSIRO in 1996 (three months) to work on microbial ecology of biocontrol agents and Professor Li Honglian visited the Australian Cotton Research Institute in Narrabri in 1998 (four months) to carry out research on biocontrol of cotton damping-off diseases.

There have been many short-term visits in both directions and these exchange visits continue to this day, usually at least once a year in each direction. These trips are essential for maintaining collaboration: they enable planning, exchange of information, reporting and social interaction.

A significant event in the long-term collaboration was the Crawford Master Class on 'Soil-borne pathogens of wheat' that was held in Zhengzhou, Henan province, in 2005. This two-week Master Class was taught by both Australian and Chinese scientists, and was attended by 21 research and extension staff from all of the major wheat-growing provinces of China. A training manual was developed (Nicol and Ryder 2005). A description of the Master Class concept and the classes that have

been held in China and other countries is found in the chapter by Nicol *et al*. (2010, pp. 97–104 this volume).

As a result of the 2005 Master Class, international collaborative research on cereal cyst nematode (CCN) in China has increased. For example, since 2005, further workshops and classes that focused on this potentially serious pest of wheat have been held in China (Riley *et al*. 2009), and these events have been supported particularly by CIMMYT, SARDI and the Crawford Fund. Several scientists from SARDI and the University of Adelaide have visited China to assist with the assessment of CCN damage to cereal crops across northern China, and with training and planning for the future. Since the 2005 Master Class, Professor Lester Burgess (University of Sydney) has been actively cooperating with several institutes in China in the identification and control of *Fusarium* disease of cereals. The Crawford Fund has supported a number of training visits and workshops in the last three years, which has further strengthened and broadened the cooperation.

Conclusions

Considerable progress has been made in scientific research and in capacity-building in soil biology and crop disease assessment and control, particularly in northern and western China, through the long-term collaborative efforts of many scientists and their support staff. Bilateral collaborations between China and Australia are continuing strongly. Progress has been made in the application of research to disease control treatments. A particular strength of the joint R&D teams is the breadth of the collaboration (i.e., number of institutes involved) and the strong continuing focus on training and the sharing of ideas, knowledge and technology.

We can ask 'Why has this cooperation been successful and so long-lived?' We think that there are two parts to the answer: firstly we have common goals (improving crop growth and yield through harnessing beneficial soil biota), and secondly we have developed trust and an ability to work together.

Acknowledgements

The authors wish to acknowledge the contributions made to the long-term collaboration by many scientists and administrative staff in both China and Australia, and thank the research team leaders, and their staff, for their commitment to advancing research and its application to improve crop growth and yield. We would like to acknowledge the contribution made by Dr Albert Rovira to the cooperation, through his initial visit to China in 1993, his stay at ZAU in 1997, his participation in the Crawford Master Class in Zhengzhou in 2005 and his continuing support through his role with the Crawford Fund.

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ATSE Crawford Fund International Master Class series on soil-borne pathogens of wheat

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Abstract

Three International Master Classes on Soil-Borne Pathogens of Wheat have been sponsored through the ATSE Crawford Fund's Master Class Program. These were held in Turkey in 2003, China in 2005 and Tunisia in 2008. A total of 63 plant pathologists from over 13 countries through the regions of West Asia, North Africa, India and China, and Australia, have participated in the program. In addition to the direct impact of the training on the knowledge and skills of individual trainees, a wide range of follow-up activities have ensured continuing benefits. Members of the teaching team have continued to mentor and support early-career trainees through a variety of measures, some of which have also been supported by the Crawford Fund.

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Introduction

Root, crown and stem diseases caused by soil-borne fungi and plant parasitic nematodes, known collectively in this paper as soil-borne pathogens (SBPs), are one of the most economically important groups of plant diseases in many crops. They are insidious diseases as they cause non-specific symptoms of the foliar plant parts, such as stunting, yellowing, premature ripening and reduced yield (Wallwork 2000; Coyne *et al*. 2007; Burgess *et al*. 2008). These foliar symptoms are commonly attributed to other causes such as drought stress, waterlogging, nutrient deficiencies and poor soil structure. Furthermore, root, crown and stem symptoms may not be obvious in a cursory examination. Consequently most are quite difficult for inexperienced pathologists, agronomists, advisors and farmers to diagnose in the field.

SBPs are also one of the most difficult groups of plant diseases to control. The pathogens can persist in root and stem residues in soil or in specialised structures in the soil matrix for one to many years. Many have a wide host range and some are able to persist by infecting symptomless crop or weed hosts. Effective control of many SBPs depends on the use of a range of measures. Crop rotation is usually a key element of most integrated disease management (IDM) strategies. Although resistant cultivars are available and effective for the control of some species or *formae speciales* of SBPs, resistant cultivars have generally proven difficult to develop for such pathogens. Other IDM measures include modification of agronomic practices such as planting rates and fertilisers to reduce moisture stress and increase plant vigour, and the adoption of no-tillage and stubble-retention practices to improve soil health and increase disease suppressiveness in soils.

Unfortunately, no-tillage and stubble retention practices can also lead to significant increases in *Rhizoctonia* and stubble-borne pathogens, such as *Fusarium pseudograminearum* responsible for crown rot of wheat (Burgess *et al*. 2001). Chemical control measures are used successfully to minimise some diseases caused by SBPs in high-value horticultural crops but are generally not available for pathogens of field crops such as wheat, for both economic and practical reasons. The success of an IDM strategy for these pathogens depends on a thorough knowledge of crop agronomy as well as the biology of the pathogen.

SBPs affect plants in cropping regions around the world. They are however often neglected in regions with limited diagnostic and research resources, regions where the focus is commonly on conspicuous foliar pathogens such as the rusts, smuts and leaf spots that can be more readily diagnosed by foliar symptoms. Furthermore, university and college courses tend to focus almost entirely on foliar diseases with root, crown and stem diseases caused by SBPs being considered only on a very superficial level, if at all.

A number of economically important SBPs of wheat are collectively of concern world-wide, causing significant losses in both developing and developed countries, particularly under drought stress. They include the cereal cyst nematodes (*Heterodera* spp. (CCN)), root lesion nematodes (*Pratylenchus thornei* and *P. neglectus* (RLN)), the take-all fungus (*Gaeumannomyces graminis* var. *tritici* (Ggt)), the crown rot fungus (*F. pseudograminearum*), the root and foot rot fungus (*F. culmorum)*, the bare-patch fungus (*Rhizoctonia* sp.) and the common root rot fungus (*Bipolaris sorokiniana*). The diseases caused by these pathogens are important in Australia where they have been studied over a long period. In contrast, there has been limited recognition of their potential importance in many developing countries, and in many other countries their occurrence and distribution have yet to be assessed. It is important to note that many of these SBPs are of economic importance in rainfed or limited-irrigation wheat cropping systems where moisture or drought stress is commonplace, including the dominant wheat-growing regions of West Asia, North Africa and northern India (Nicol and Rivoal 2008).

Background to the Crawford Fund Master Class program

The 'master class' training concept was conceived by Prof. Bruce Holloway, then of Monash University, as the key training initiative on microbial and molecular genetics embedded in Australian Centre for International Agricultural Research (ACIAR) projects on bacterial wilt (PN9015 and PN9452). Three classes on microbial and molecular genetics were held between 1992 and 1994. These classes were designed as intensive three-week teaching programs with an emphasis on interactive teaching techniques, a variety of teaching aids and alternating tutorial room and laboratory sessions. In 1994, the President of the Academy of Technological Sciences and Engineering (ATSE) invited Fellows to suggest new activities for the Academy. Prof. Holloway proposed that the Academy set up a program of master classes to train scientists and technologists in the South-East Asian region in areas of technology relevant to the Academy's objectives.

Following discussions at committee level, Prof. Holloway decided that the focus should be on agricultural biotechnology and the president suggested that the Crawford Fund, a part of the Academy, was an appropriate administrative organisation for the master classes. Prof. Holloway subsequently became the Coordinator for the Master Class Program, a position he held until 2004. During his tenure he was responsible for coordinating 24 classes covering a wide range of scientific topics, as well as research management in agriculture (Metcalfe *et al*. 2005). Prof. Holloway raised over A$2M from external agencies to support this training. Dr Paul Ferrar was the coordinator from 2005 to 2008, during which time a further seven classes were held. Dr Eric Craswell succeeded Dr Ferrar as coordinator in 2008. A total of 630 trainees were enrolled in the 31 classes presented through the program.

International Master Classes on soil-borne pathogens of wheat

In 2001, the CIMMYT International Pathology Program on soil-borne pathogens was moved from CIMMYT headquarters in Mexico to the regional office in Turkey where Dr Julie Nicol was appointed as the Senior Plant Pathologist. The goal was to work collaboratively with the Turkish Ministry of Agricultural and Rural Affairs (TAGEM), with responsibility for fostering an awareness of the nature,

and potential economic importance and control, of SBPs in the rainfed wheat production systems of West Asia and North Africa (WANA). One of the key responsibilities of the CGIAR (Consultative Group of International Agricultural Research) centres such as CIMMYT is to provide opportunities for scientists in national programs to develop their research skills.

As in Australia, Dr Nicol realised that SBPs were a major constraint to wheat productivity in the rainfed wheat production systems of the WANA region. It was also realised that the small number of experienced researchers was one of the major limitations to the diagnosis and control of these diseases. Consequently, an urgent need for intensive training in the diagnosis, biology and control of these diseases as well as survey and crop loss assessment techniques was identified. It was clear that Australia had particular expertise and experience in research on SBPs of wheat and the Crawford Fund's Master Class Program was an appropriate and proven platform for the efficient training of wheat pathologists from the predominantly rainfed wheat cropping regions of WANA.

Dr Albert Rovira, formerly Chief Research Scientist in Soil Biology at CSIRO, Adelaide, a member of the South Australia Crawford Fund Committee, was approached for support. Following discussions with the Committee and its chair, Dr John Radcliffe, and the coordinator of the Crawford Fund's Master Class Program, Prof. Holloway, it was agreed that The Fund would be the key sponsor of the first master class on SBPs of wheat to be held in Turkey in 2003. In collaboration with Dr Rovira, additional support was obtained from TAGEM,ACIAR, the International Centre for Agricultural Research in the Dry Areas (ICARDA), the Grains Research and Development Corporation of Australia (GRDC) and the Kirkhouse Trust in the United Kingdom. Dr Rovira also helped to recruit the Australian teaching team for the class, including *Fusarium* specialist and pathologist Prof. Lester Burgess (The University of Sydney), wheat pathologist and geneticist Dr Hugh Wallwork (SARDI)) and nematologist Dr Ian Riley (University of Adelaide and SARDI). In addition, Dr Rovira provided valuable support and advice during the development of the class program and training manual.

Class objectives

The class was designed, through a series of modules, to enhance the ability of trainees to:

- understand the biology, pathology and population dynamics of important SBPs, viz., parasitic nematodes and root, crown and stem rotting fungi of cereals
- isolate, extract and identify these SBPs
- understand the methodologies to scientifically establish losses caused by one or several of these SBPs
- understand the control options for the different SBPs, with an emphasis on the use of host resistance and other environmentally friendly control methods
- understand the principles of incorporating resistance to SBPs into a cereal-breeding program
- understand the application of molecular biology both in the identification of the pathogens and the breeding of disease-resistant germplasm against the identified SBPs
- contribute to increased research capacity building in their home organisation
- further develop their research management and personal skills
- establish a regional network of pathologists with key pathologists in the region who may work on these SBPs.

The class included tutorials, discussions, extensive practical laboratory sessions and field excursions. The emphasis was to provide practical solutions to real problems encountered in the trainees' home countries.

Classes in Turkey, China and Tunisia

Three International Master Classes on SBPs of wheat have been held. The first was in Turkey in June 2003 (Fig. 1) coordinated by Julie Nicol (CIMMYT) and hosted by TAGEM under the then Director General Dr Hasan Ekiz. The 23 trainees of the class came from the WANA region, with representatives from Afghanistan, Iran, Morocco, Syria, Tunisia and Turkey, with the addition of trainees from Australia, India, Kazakhstan and Uzbekistan. Besides the Australian teaching team, contributions were made to the master class by researchers from TAGEM and Cukurova University,

Figure 1. First Master Class on Soil Borne Pathogens of Wheat, Eskisehir, Turkey, 14–29 June 2003

Figure 2. Second Master Class on Soil Borne Pathogens of Wheat, Zhengzhou, China, 9–20 May 2005

CIMMYT, ICARDA and INRA, France. Since the class, national projects have been implemented in Turkey, in collaboration with CIMMYT, on cereal nematodes and dryland root rots. These projects have clearly demonstrated the importance of SBPs in the rainfed wheat systems in Turkey (Hekimhan *et al.* 2004; Toktay *et al.* 2006; Sahin *et al.* 2008; Tunali *et al.* 2008; Elekçioğlu *et al*. 2009). A key aspect of this work has been the identification and distribution of promising sources of resistant germplasm to a number of regional collaborators where SBPs are known to be economically important (Nicol *et al.* 2007, 2008).

Figure 3. Third Master Class on Soil Borne Pathogens of Wheat, Tunis, Tunisia, 28 April – 9 May 2008

Figure 4. Dr Albert Rovira with Drs Julie Nicol and Li Honglian (left and right of Albert) and a group of trainees involved with fieldwork at the Second Master Class on Soil Borne Pathogens of Wheat, Zhengzhou, China, 9–20 May 2005

The success of the first class provided the impetus for two further classes, the second in China in 2005 (Fig. 2) and the third in Tunisia in 2008 (Fig. 3). The design of these classes was based on the format devised for the Turkey Master Class but with some modification of content to suit local circumstances.

The China class was jointly coordinated by Drs Maarten Ryder (CSIRO) and Julie Nicol, and held at Henan Agricultural University in Zhengzhou.

Dr Albert Rovira (Fig. 4) and Dr Ryder, having had much experience in China in their research careers, played a pivotal role in identifying and coordinating local teaching staff in collaboration with eminent plant pathologist Prof. Tang Wenhua, from China Agricultural University in Beijing. As before, the major donor was the Crawford Fund, with additional support from two international and seven local donors. The local logistics were organised by Prof. Li Honglian of Henan Agricultural University in close collaboration with Dr Ma Ping from the Institute for Plant Protection, Hebei Academy of Agriculture and Forest Sciences. As China is a large and diverse wheat-producing country, the class was limited to 21 trainees from 13 Chinese provinces. The Australian teaching team led the class with important contributions from leading Chinese researchers. The class was rated highly by the trainees. One of the key outcomes was the unexpected finding of high levels of the cereal cyst nematode (CCN) in Henan and three adjacent provinces (Anhui, Hebei and Shandong).

The third class in Tunisia in 2008 was hosted by the Institut National de la Recherche Agronomique de Tunisie (INRAT) under the Director General Dr Amor Chermiti and coordinated by Julie Nicol in collaboration with three INRAT staff, Drs Gargouri, Saleh and Kachouri. Again, the Crawford Fund was the main sponsor together with two CGIAR centres, CIMMYT and ICARDA, and the Kirkhouse Trust. The 20 trainees came from Algeria, Australia, Iran, Kazakhstan, Libya, Morocco, Syria, Tunisia and Turkey. The format was similar to the earlier classes and the core Australian teaching team remained unchanged. The INRAT researchers mentioned above and others from the ICARDA-CIMMYT Wheat Improvement Program and INRA also contributed.

Class program

The training program for each class included 20–40 minute tutorials on specific topics using a variety of teaching and learning techniques, alternating with laboratory sessions and including two days of field studies. The tutorials were designed to be highly interactive and draw on the experiences of the trainers and the trainees. The varied program was essential to maintain the enthusiasm of both trainers and trainees. It also enhanced the understanding of important concepts through a process of consolidation and reiteration of key ideas and techniques in different contexts.

An introductory session considered the key features of SBPs and was designed to emphasise the interactive and inclusive nature of the teaching and learning process and ensure that each trainee was contributing. This was a critical session as many trainees come from cultures where teaching focuses more on imparting information and involves little or no discussion, problem-solving or critical analysis. An introduction to the biology and control of the key diseases followed to provide a background for the first field trip on either day two or three. The first field trip provided an opportunity for trainees to identify symptoms in the field (Fig. 5), collect samples for laboratory diagnosis (Fig. 6) and discuss field survey procedures. The second trip involved inspection of field trials designed for crop loss assessment purposes or the evaluation of host resistance.

The isolation or extraction of pathogens, their purification, identification and enumeration were key aspects of the laboratory sessions (Figs 7 and 8), and complemented discussions on the biology and control of the individual pathogens. The importance of accurate identification and diagnosis was emphasised.

The development of IDM strategies with an emphasis on rotation and resistant cultivars was a recurrent topic throughout the class. In the class in Tunisia, the impact of no-tillage on diseases such as crown rot was considered extensively together with a presentation on no-tillage and the critical role of rotation in no-tillage farming systems

The use of resistant cultivars is a key component of IDM strategies for control of several key pathogens, especially CCN, RLN and crown rot fungi. Several sessions on the principles underlying the

Figure 5. Dr Julie Nicol discussing symptoms of cereal cyst nematode during a field trip in the Master Class in Tunisia, 2008

incorporation of resistance into adapted cultivars were included. Screening methods and the use of molecular markers were discussed in detail in combination with laboratory, greenhouse and field studies. The crucial role of international centres in providing germplasm in initiatives between cooperating countries and the CGIAR (as demonstrated in the CIMMYT-led SBP network's multi-location screening of germplasm for resistance to SBPs, Nicol *et al*. 2007) was emphasised.

Professional development of trainees was considered and the establishment of research networks encouraged. The classes concluded with small groups undertaking case studies. Their reports included formal Power-Point presentations.

Evaluation and feedback provided by trainees through formal surveys has been highly positive. Moreover, the outcomes and benefits of these classes extend well beyond the direct impacts of the class on the understanding of these diseases and the technical skills learnt by the trainees. Examples of these are discussed below.

Two early-career Australian researchers were also included as trainees in the classes held in Turkey and Tunisia. They provided peer support to other young trainees as well as assisting the teaching team (Fig. 8), thereby enhancing the effectiveness of the learning experience. The four trainees and the Australian grains industry will benefit from the international exposure and networking opportunities, as well as a broader understanding of SBPs. Furthermore, in collaboration with local colleagues, one of these trainees completed a survey of *Fusarium* species associated with wheat in Turkey as part of her PhD (Bentley *et al*. 2006).

Class outcomes

The types of extended outcomes of the classes can be illustrated by reference to the second class held in China in 2005.

Following identification of high levels of CCN during the first field trip, a survey of CCN in wheat fields was conducted over the following weekend by the teaching team and trainees. The survey conducted in Henan and three adjacent provinces revealed

Figure 6. (L to R) Dr Gargouri, Noel Knight, Dr Saleh and Phillip Davies collecting take-all samples during a field trip in the Master Class in Tunisia, 2008

Figure 7. Prof. Lester Burgess and trainees in the laboratory during the Master Class in Tunisia, 2008

Figure 8. Phillip Davies, an Australian trainee, assisting other trainees with fungal isolation procedures during the Master Class in Tunisia

that CCN occurred in 90% of the fields at population densities greater than anticipated and likely to cause significant yield losses. This led to efforts by Master Class trainees, their supervisors and national experts to survey the distribution of CCN more widely in wheat-producing provinces, determine population densities, characterise pathotype and genetic diversity, assess yield loss and test the suitability of available resistance genes. Provincial-level support has since been secured and CCN is likely to be recognised as a pest of national importance in China (Li *et al*. 2007; Peng *et al.* 2007; Riley *et al*. 2007).

A further training workshop, specifically on CCN, was held in Baoding, Hebei, in 2006 with two of the Master Class team (Julie Nicol and Ian Riley) working together with Dr Sharyn Taylor (formerly of SARDI) as facilitators. The South Australia Crawford Fund Committee provided the direct financial support to make this workshop possible. In 2007 and 2008, CCN review and planning workshops held in Beijing and Xining, Qinghai, were coordinated by Prof. Peng Deliang (CAAS, Beijing) representing the CCN Network in China that grew from the Master Class and CCN workshop. CIMMYT and Australian researchers provided an international perspective on CCN at these meetings. Through these initiatives the CCN research program has been expanded significantly in China. Most recently two large national projects with CIMMYT collaboration have been funded in China to address CNN (Nicol, pers.)

Most of the post Master Class outcomes occurred through the CCN network in irrigated winter-wheat producing provinces (Riley *et al.* 2007). As it is well recognised that damage by CCN is increased under rainfed or stressed wheat production systems, it was decided that some support and research would be directed to the more marginal spring wheat growing areas in northern China. The South Australia Crawford Fund

Committee enabled Dr Riley to provide individual follow-up support to three Master Class trainees in Gansu and Qinghai in 2007. With funding from a Cheung Kong Endeavour Australian Fellowship, Dr Riley also spent the second half of 2008 in China undertaking field studies on CCN in spring wheat in high-altitude villages of Qinghai, in collaboration with a former Master Class trainee. The goal was to examine the impact of rotational crops on CCN and spatial patterns in CCN populations with a view to developing recommendations on control and future research options. Findings from these studies indicated that natural biocontrol is the strongest determinant of CCN densities and, where biocontrol is not operating effectively, damaging populations still occur, irrespective of the rotation. Time was also devoted to reinforcing training provided by the Crawford Fund programs.

Following each Master Class, selected trainees were recommended for further specialised training. The New South Wales Crawford Fund Committee sponsored a three-month visit for two trainees of the China Class to the Fusarium Research Laboratory at The University of Sydney in 2006 to study with Prof. Lester Burgess. This involved detailed work on *Fusarium* taxonomy and identification, and the biology and control of *Fusarium* diseases of cereals and other crops in wheat farming systems. This led to changes to the PhD programs of the visiting students and Prof. Burgess becoming a co-supervisor for each student. In 2007, Prof. Burgess visited China for two months and attended their final seminars and defence of thesis. He also undertook a survey of *Fusarium* species in Gansu. In addition, he presented a two-week workshop on soil-borne fungal plant pathogens at Gansu Agricultural University (GAU) for six trainees representing three universities and the Plant Protection Research Institute in Beijing. This workshop, based on the master class concept, was sponsored by the New South Wales Crawford Fund Committee. The tutorials were also attended by 20 undergraduate and postgraduate students from GAU. One of the original trainees who came to Sydney in 2006 attended advanced English training in Beijing and was awarded a Chinese Government Fellowship in 2008 for research at the CSIRO in Adelaide. Her research is being supported in part by the South Australia Crawford Fund Committee.

The follow-up activities outlined above provide examples of the long-term benefits and positive impacts at both country and personal level, of the Master Class program.

A recent study by the Centre for International Economics (CIE 2009) shows that with an investment of about $200 000 the 2005 Master Class on cereal cyst nematode in China potentially yielded benefits of $20 million by bringing forward by one year the benefits of CCN control.

Conclusions

The success of these Master Classes and the continuing research and training activities are a testament to the foresight and support of key Australian scientists such as Drs Rovira and Radcliffe. They were aware of the importance and difficulty of working with SBPs of wheat and strongly supported the establishment of these Master Classes.

Another key to success of the three classes has been the leadership provided by the directors of the Crawford Fund Master Class program (Prof. Bruce Holloway and Dr Paul Ferrar) and the in-kind time support of SARDI, The University of Sydney and The University of Adelaide in allowing their staff to continue as the teaching team, providing consistency across classes. An important part of this support has been the development of a teaching manual, which will be published shortly and will serve as a basic resource for future training programs.

It is important to note that the long-term impact of any one class is dependent on the follow-up research, networking and training activities. The Crawford Fund has played a pivotal role in supporting further training activities and workshops for key researchers identified from the Master Classes. The cooperation between CIMMYT, ICARDA and partners in the host countries has also played a critical role for these on-going activities, and in the establishment of new research programs.

Master Classes funded by the ATSE Crawford Fund have also been a catalyst to enable important forums to occur, such as the First International Cereal Cyst Nematode Iniative meeting recently held in Turkey with more than 60 participants representing 20 countries. With a dedicated editoral team a published proceedings has been produced (Riley *et al.* 2009) with more than 10 papers representing former attendees to the various Master Classes.

Recent publications by former trainees (e.g. Bentley *et al.* 2006; Toktay *et al.* 2006; Sahin *et al.* 2008; Tunali *et al.* 2008) illustrate the strength of the research projects fostered through the classes and follow-up activities. Key findings have been published from Turkey, Iran, Tunisia, China, India and Australia.

Food security and sustainable crop production are the two most urgent issues to be resolved in rainfed marginal wheat production systems in semi-arid regions. These Master Classes clearly address these issues. The development of diagnostic and research capacity in these regions is leading to wider recognition of the economic importance of SBPs of wheat, and appropriate control measures for them. As these pathogens commonly cause losses of 20–40%, significant gains in yield can be achieved through the use of resistant cultivars, crop rotation and other measures. Thus effective control has a major impact on food security in these regions, irrespective of other inputs. Furthermore, the classes address the problems of poor soil structure, low organic matter and fertility, and susceptibility to wind and water erosion. No-tillage, soil health and crop rotation are discussed in detail together with a consideration of the biology and control of stubble-borne diseases. This is an area where Australian researchers have long experience.

Continuation of these classes and their complementary activities will provide significant future benefits to developing countries as well as Australia. The authors are indebted to the Crawford Fund for its continuing commitment to this vision.

Acknowledgements

The financial and in-kind support of the following international and Australian organisations is gratefully acknowledged: ACIAR, ATSE Crawford Fund, Chueng Kong Endeavour Australia Awards, CIMMYT, CSIRO Adelaide, GRDC, ICARDA, SARDI, The Kirkhouse Trust (UK), The University of Adelaide, The University of Southern Queensland and The University of Sydney.

The financial and in-kind support for the classes from local donors in each of the three host countries is also gratefully acknowledged: Turkey — Ministry of Agricultural and Rural Affairs; China — China Agricultural University, Henan Agricultural University, Henan Plant Protection and Quarantine Station, Henan Society for Plant Pathology, National Engineering Research Centre for Wheat, Syngenta China Investment Co. Ltd, Bayer CropScience (China) and Xinjiang Kangzheng Agri-science and Technology Co. Ltd; Tunisia — Institut National Research Agronomique.

The leadership and support of senior wheat management staff of CIMMYT and ICARDA, specifically Drs S. Rajaram, Hans J. Braun, Amor Yahyaoui and Richard Brettell, is gratefully acknowledged. The continuing financial and staff support provided by these organisations has enabled the senior author to devote time to the initiation, coordination and follow-on activities of the classes.

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Plant and soil health and farming systems—a farmer's perspective

Neil Smith[1]

Abstract

A series of technical innovations, commencing in 1900, has enabled Australian farmers—typically operating on inherently infertile soils—to raise grain yields threefold. In the last round of innovation, in the last two decades of the 20th century and in which Albert Rovira had a prominent role, the management of root disease and minimisation of tillage were key elements. The diminishing availability of external inputs poses a serious challenge for the future. Pasture legumes will have an expanded role in providing nitrogen, but unfortunately there is no corresponding source of phosphorus.

Introduction

Australian soils are inherently infertile. Since European settlement, the meagre fertility status of the country's soils and adapted flora has been vigorously exploited and or discarded.

The ingenuity of farmers with adaptive and advancing science and technology has enabled the soils, which are naturally rain-fed, to yield at increasing levels. These trends were shown up until the end of the 1950s by the classic diagram of Donald (1964, 1965) (Fig. 1).

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Technical improvement has continued since then. The outcome on Australian grain farms in the period 1977–1978 to 2001–2002 has been that multifactor productivity growth (growth in output relative to the combined contribution of key inputs, usually labour and capital) has increased, on average, by around 3.3% per year (Angus 2001). Explanations for the superior performance of the cropping industries include significant changes in cropping technology encompassing crop varieties with improved resistance to disease, more effective use of as well as improvements to fertilisers and pesticides, and greater labour productivity (Productivity Commission 2005).

The Rovira legacy

The adaptation of results from the long-term trial work started by Rovira and continued by Roget at the low-rainfall site at Avon, north-west of

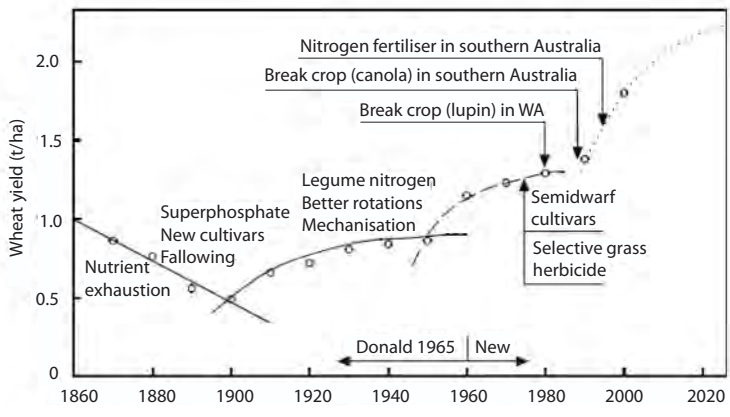

Figure 1. Average grain yields (for each decade) of Australian wheat crops. This graph was generated by Angus (2001) using data from Donald (1965) and ABARE. The dotted line represents yields projected from the increase during the 1990s.

Adelaide, has been responsible for the major positive changes in farming systems in the critical 1980s and 1990s. There were significant technological developments in tillage design, herbicides and fertiliser use. Rotations were developed to exploit these technologies. This, combined with knowledge emanating from the Avon work, generated yield increases as root diseases were contained. Essentially, the Rovira legacy has consisted of recognising that rhizoctonia could be somewhat controlled by deep cultivation, desirably three weeks before sowing to minimise the impact of the fungus. Their research also showed that long-term retention of trash resulted in the development of 'suppressive soils' allowing no-till or direct drill yields to 'catch up' with those from conventional soil preparation, but with the added advantage of minimal disturbance of Australia's fragile soils. Hence the retention of trash—so critical to the fertility status of southern Australian soils—became accepted by farmers as essential.

The research undertaken by Rovira and Roget was among the most important scientific work that has been adopted by farmers since the selection and introduction of medics for the pasture phase of the ley-farming system developed in the 1950s.

But where do we go in the future?

Pastures (legume dominant) in farming systems

The climate is predicted to become increasingly drier and hotter in the southern latitudes. Fossil-fuel-derived inputs have become much more expensive through increasing scarcity of oil that will continue to worsen in the future. Agriculturists will find that unless they can employ all that science can provide them, together with an accurate knowledge of the real cost of each unit of production (tonnes per hectare), they will face a very hard time even in more favoured areas. We, as farmers, have been able to profitably overcome the natural poverty of Australia's soils in recent years only because oil-based products were so cheap.

In South Australia, not since the run of dry years from the late 1800s or the great depression of the 1930s, have we as farmers had to face a challenge such as that ahead.

The rapid changes in the operating environment have just shifted to a higher gear!

In my view, a key feature of a failed cropping operation in the future will be poor management of the pasture phase. Unfortunately, it is rare to see well-managed pasture in areas below the 375-mm rainfall isohyet. By inference, these areas have no future without radical change in commercial systems development and adjustment. There will be little alternative to improved livestock production systems in these regions, based on managed or native pasture systems with only 'opportunistic cropping'.

With the virtual doubling of the price of applied nitrogen (now $2/unit for urea-derived nitrogen), no longer can all cereal nitrogen needs be applied 'from the bag' because of the increasing risk associated with variable climate. At the current cost of nitrogen, farmers will quickly accept that a well-managed pasture (legume dominant—grasses removed) will be the norm. Sowing of the crops on 20–40% of the area of a farm would allow carrying a legume pasture simply as a source of nitrogen. Grazing of that phase will become complementary to nitrogen production, not an essential revenue source in its own right as it has been in the past. All types of possibilities surround the need to plan for plant-nitrogen as the principal source of nitrogen for non-legume grain production. Pastures just happen to be the most practical approach.

Work by Ed Hunt, an agricultural consultant from Eyre Peninsula, indicated that even before the rapid increase in the price of nitrogen fertilisers, continuous cropping, even in highly favoured 450-mm zones, may not be as effective as having 15% or more of the farm's area sown to a well-managed pasture.

On our farm, the total cost of growing wheat is now $540 per hectare on brown manure (residue from grazing pasture legumes) or $481 on good pulse stubble with 35 units of nitrogen from industrial sulphate of ammonia. With a wheat crop average yield that has dropped from 4.3 tonnes per hectare during 1995–2001 to 3.0 tonnes per hectare on brown manure and 2.7 tonnes per hectare on pulse stubble in the drier seasons of 2002–2007, to break even at current averages a price of $180 per tonne at the farm gate has to be achieved. That price may have been achievable as at the date of this symposium, but what about next year? The expected price of a unit of nitrogen from industrial-grade sulphate of ammonia has been assessed at $1.50. If urea is to be used ($925 per tonne delivered and spread), then the return for wheat on pulse stubble would need to be $497 per tonne.

The advantages of growing nitrogen by legumes are:

- The cost is 'internalised' (equivalent to 80 units of nitrogen at $1.50 per unit, valued at $120 per hectare).
- Sheep are grazed on brown manure for prime lamb production.
- There is much less financial risk due to less up-front outlay.
- The area of pulses, a high-risk crop, is kept to a minimum.

The other nutrient to rapidly increase in price is phosphorus. The on-farm cost of applied phosphorus is now about $6.40 per unit, with 3–4 tonne per hectare wheat crops needing 9–12 units per hectare as a minimum—about $75 per hectare. Most Australian soils are very deficient in phosphorus. Our farm soils contain roughly 40–50 units of Colwell P that is available to plants. We will be able to survive for a few years on less than optimal dressings. Not so many other areas of South Australia, particularly western Eyre peninsula where phosphorus is rapidly locked up on the highly calcareous soils.

Conclusion

Stresses are appearing in Australia's grain industries, but farmers on the better lands will continue to flourish. The world population is approaching seven billion. Grain prices will be volatile, making it harder for the exporting agriculturist to manage a successful business. Good farm accounting and marketing knowledge will be essential.

We have probably passed through the halcyon days from the late 1980s to 2001 when fertiliser and fuel were cheap. (In 2001, the average diesel price was 41c per litre, but at the time of this symposium it is $1.25 per litre.) Those days were a time when recent technology and science outcomes had combined to provide the grain farmer with massive opportunities. There will still be new technologies and scientific discoveries, but it is my observation that over the past forty years the excellent work from the public scientific sector has started to decline. Some of the 'bells and whistles' from the private sector on new technologies may be obscuring clear thinking by farmers about the underlying principles of agriculture.

Albert Rovira undertook his work at the right time for agriculturists to reap the benefits. There is still a challenge in the future for rhizosphere scientists.

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Abstracts

New technologies for more in-depth understanding of the soil and rhizosphere communities

James M. Tiedje[1,2], WooJun Sul[1], Stephan Ganter[1], Mary Beth Leigh[1], Deborah Yoder-Himes[1] and James R. Cole[1]

Much of Earth's terrestrial productivity depends on the ability of plants to convert the energy from sunlight into useful organic compounds for food and now for liquid fuels. Plants do not live in isolation but in concert with microbes around their roots, on their leaves and internally (endophytes). As we are driven to lower-cost, more productive and sustainable primary production systems, we need these communities to live in harmony and at maximum efficiency.

Can we 'manage' the plant–microbe associations, especially those in the rhizosphere, to enhance sustainability of productivity? This will be especially important in any new cellulose-to-biofuel economy where energy crops would most likely be grown on marginal lands, and to maximise energy gain and ensure carbon neutrality.

New molecular technologies, especially the 'omics', provide the opportunity to understand microbial communities and their interactions in much great detail. This is illustrated by the insight into soil and rhizosphere communities provided by new high-capacity sequencers. The ecology and dynamics of particular populations, and which genes and their regulators are expressed in contrasting niches, can be better understood. The patterns and diversity of *Burkholderia* and *Pseudomonas* populations in rhizospheres provide good examples of this.

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Trophic cascade and decreased survival and growth of *Pinus strobus* seedlings in microcosms caused by fungivory on mycorrhizae

Sina M. Adl[1,2] and Melanie L. Wilson[1]

Can fungivory post-germination during mycorrhization affect seedling growth and survival? Most studies on mycorrhizae have been carried out on older seedlings with established mycorrhizae. We have focused on early seedling growth. We selected eastern white pine (*Pinus strobus*) which was germinated in soil microcosms, reconstructed using a protocol similar to one used in the Setälä laboratory. A full factorial design with five treatments, each randomly replicated in ten trays (n=10), was established in a controlled-environment greenhouse. Treatments were:

a) no ectomycorrhizae

b) three species of ectomycorrhizae (EM)

c) EM and enriched with fungivorous nematode culture (Nf)

d) EM, Nf and enriched with microarthropods (MA)

e) EM and MA.

Seedlings were destructively sampled at four months or at seedling death (< 4 months). Data were analysed with ANOVA and multivariate procedures and results were compared across treatments for seedling survival rate, age at death, growth, mycorrhization and animal abundance and composition. Fauna were grouped into functional categories. Seedlings without ectomycorrhizae did not survive to four months. Fungivory by nematodes or microarthropods (treatments c and e) reduced mean (%) mycorrhizae on roots. Enrichment with both fungivorous nematodes and microarthropods (treatment d) decreased fungivory and permitted mean (%) ectomycorhhizae levels similar to treatment with no fungivory (treatment b).

Canonical scores plot in discriminant analysis clearly separated treatments by mean (%) root tip colonisation. Some functional groups correlated with treatment response. In addition we observed:

- a trophic cascade, confirming an earlier microcosm study by the Setälä laboratory, and expanding the results to germinating seedlings during mycorrhization
- that fungivory in the rhizosphere can decrease mean mycorrhization levels, and affects both seedling survival and growth.

Comparison of animal abundances and mycorrhization levels confirms that seedlings less than one year old affect their rhizosphere. Thus rhizosphere food web structure has consequences for seedling growth and survival through mycorrhization level.

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Co-opting the endorhizosphere

Christopher M. Franco[1,2]

The endorhizosphere of cereal crops contains a diverse microbial population as revealed by microscopy, culture-independent techniques and isolation of pure cultures. Our study focussed on endophytic actinobacteria—the rationale being that actinobacteria are recognised as prolific producers of bioactive compounds such as plant growth regulators and antifungal agents; their mycelial growth promotes colonisation and, as endophytic partners, they work from within the plant where they are protected from environmental stresses and competition from soil microflora. Actinobacteria isolated from surface-sterilised roots of young healthy cereal plants were identified and tested for their ability to suppress root diseases *in planta* in greenhouse and field trials. Selected strains improved grain yields by 5–60%, compared with untreated controls, in multi-location field trials across Australia over the past five years. Isolates effective in controlling root diseases belonged to *Streptomyces*, *Microbispora* and *Nocardioides* genera. Most isolates were capable of suppressing multiple fungal pathogens including *Rhizoctonia solani*, *Gaeumannomyces graminis* var. *tritici* and *Pythium* spp. through induction of systemic resistance and antibiotic production. These actinobacterial inoculants are compatible with farm management practices such as the application of fertilisers, herbicides and pickling agents, and can withstand on-farm environmental conditions.

Colonisation of plants by actinobacterial endophytes, studied using confocal microscopy and T-RFLP, revealed an early colonisation of external surfaces and within plant tissue, especially below the epidermal layer of roots and at lateral root junctions. The inoculant, added initially as a seed coating, does not radically disrupt the endemic endophytic diversity and was found to colonise wheat plants for up to 12 weeks after germination. During this early growth period the actinobacterial partner increased plant emergence and root and shoot length.

These beneficial interactions result from an intimate relationship between endophytic actinobacteria and host plants. This partnership is a new facet of microbial ecology that enables a biological means to significantly increase grain yields and reduce the reliance on chemical fungicides and petrochemical-derived fertilisers. This technology is sustainable and has widespread application for broad-acre agriculture, pasture production, horticulture and floriculture.

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Varietal differences in cotton—belowground

Oliver G.G. Knox[1,2,3], Vadakattu V.S.R. Gupta[3,4,5], Kellie Gordon[2], Richard Lardner[4], Paul Harvey[4] and Marcus Hicks[4]

Rhizosphere microbial diversity is known to differ between various crop species. There is also reported evidence of varietal differences in rhizosphere bacterial diversity within crops such as wheat. During work conducted between 2002 and 2006 on the potential for genetically modified cotton to affect microbiota in Australian soils it became apparent that crop variety can influence microbiology associated with the rhizosphere.

A project was commenced in 2006 to determine the influence of current commercial cotton varieties on their associated rhizosphere microbial populations and functions. Rhizosphere samples for 15 cotton varieties were collected from field experiments conducted at the NSW DPI Myall Vale Research Station, Narrabri, during the 2006–2007 and 2007–2008 seasons and analysed for microbial biomass and activity, catabolic diversity of microorganisms (MicroResp®), bacterial diversity and populations and activities of nitrifying and free-living N-fixing microorganisms using culture-based and DNA techniques.

Analysis of microbial biomass and activity indicated large seasonal fluctuations and showed that differences between varieties were non-significant. The fluctuations in microbial biomass values observed throughout the season were taken as an indication of the influence of changes in exudation, as plants redirected resources according to physiological requirements.

Bacterial population analysis based on 16SrDNA-DGGE indicated a strong association between varieties and families of varieties based on specific rhizosphere bacterial populations early in the season (63 days after sowing), which disappeared later in the season (176 days after sowing). MicroResp® results over the two years showed clear varietal influences on the capability of the rhizosphere communities to utilise specific compounds. Canonical analysis of catabolic profiles indicated a varietal-based response of rhizosphere microbiota to specific hexose sugars and amino acids.

Data on ammonia oxidising populations and nitrification rates indicated significant differences between varieties but trends varied between the two seasons. We also observed varietal differences in the capability of rhizosphere soils to carry out non-symbiotic N_2 fixation.

Overall, our results show that cotton varieties are altering the soil microbiology in their rhizospheres and, in turn, the levels of functions that these microbes carry out. Through developing a better understanding of what drives these changes and how wide spread they are, it should be possible in the future to develop lower input and more efficient input systems that capitalise on varietal selection. Further research is required before field recommendations can be made.

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Root damage constrains autumn–winter pasture yield and farm productivity

Richard Simpson[1,3], Alan Richardson[1], Ian Riley[2] and Alan McKay[2]

Root damage on subterranean clover as a result of root-rot pathogens has been variously described as a substantial problem for clover yield or as an intermittent problem that can be severe in certain years. It is likely, however, that the true cost of root damage is not fully appreciated because sub-lethal damage to pasture roots is hidden to casual observation and the costs to production tend to go unrecognised.

Bioassays using subterranean clover (cv. Woogenellup) or annual ryegrass (cv. Safeguard) were used to investigate the potential for root damage at four field sites in NSW and to estimate the cost of damaged roots for plant growth in autumn–winter. Substantial seedling losses and damage to roots of clover and ryegrass were observed. Autumn–winter plant growth was negatively related to the extent to which roots were damaged. Root damage limited clover growth by 21–36% and ryegrass by 10–45%, depending on the site.

Simulation modelling was used to estimate the cost to a sheep enterprise of such a constraint to autumn–winter pasture productivity. It was estimated that a well-managed enterprise would forego between 25% and 58% of potential net farm income given the levels of root damage measured in this study. Root damage was also predicted to limit gains in farm income that would normally be expected when stocking rates are lifted.

DNA assays for root pathogens and some beneficial organisms were employed to quantify the microorganisms associated with damaged roots. Increasing levels of *Phytopthora clandestina* and *Pythium* clade F associated with increasing clover root damage at three sites but lacking association at the fourth, and with grass root damage, indicated a need to also develop DNA assays for other root pathogens.

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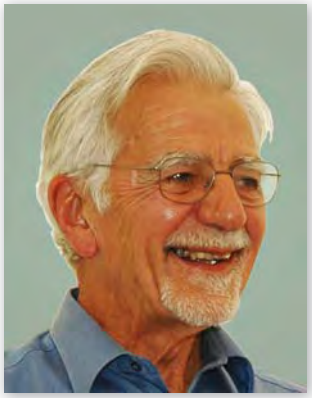

Biology of the rhizosphere: progress and prospects for the future

Albert D. Rovira[1,2]

Abstract

As the final speaker of the day and as a person who became interested in the rhizosphere back in 1951 before many of the today's speakers were born, and following speakers who are actively involved in rhizosphere research, I had a challenge in preparing this talk.

I believe that, despite all the modern techniques that are available and the reliance of scientists on computer searches for past information, knowledge of the history of a subject enables scientists to avoid the repetition of past research and leads to faster progress.

The big breakthroughs in rhizosphere research have often followed the application of techniques developed in other areas of science. Examples of this are the application of paper chromatography to determine the composition of root exudates, the application of transmission and scanning electron microscopy and the use of DNA techniques to detect and quantify cereal root pathogens in Australian soils. In this paper I will discuss the outcomes of the basic research to understand the biology of the rhizosphere and how this knowledge was extended to soil-borne root diseases, and finally the application of this knowledge to develop sustainable rain-fed farming systems.

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As this symposium is sponsored by the ATSE Crawford Fund dedicated to increasing food production in lesser developed countries, I will finish my talk with a few suggestions on how some of the results of rhizosphere research may be used to further the work of the Crawford Fund.

Introduction

This symposium in my honour came as a great surprise and I am deeply honoured to be part of it—to have reached 80 years of age is largely genetic, life style and luck, but to have worked on the rhizosphere was a matter of fate. It resulted from coming across three reviews on the topic shortly after I graduated from the University of Melbourne in 1948. These were reviews by three pioneers in this field: Starkey (1931), Katznelson *et al.* (1948) and Clark (1949). I was interested in soil microbiology and figured that if I was to work on this for my doctorate at the University of Sydney starting in 1951 and possibly later in my postdoctorate career, then rhizosphere microbiology was preferable to general soil microbiology because improved knowledge of the rhizosphere would be a more direct route to improved plant performance.This is not a review of the subject, but rather a personal journey through the rhizosphere written in the first person, based on my experiences and interests over the past 50 years and with an emphasis on Australian research, much of which is published in Australian journals often overlooked overseas. Over the years I have worked in many laboratories around the world, but spent most of my time in the CSIRO Division of Soils, Adelaide. Visiting scientists, post-doctoral fellows

and post-graduate students have all made significant contributions to my research. I have also had strong support from Ted Ridge, the late Andrew Simon, Greig Whitehead, Neil Venn and David Roget. I also want to thank Mr Robin Manley for allowing us to conduct field trials on his farm at Avon in South Australia for some 30 years. Professor Jim Cook from the USDA and Washington State University and I have talked and worked together over many years and it is interesting to note that although we started with very different backgrounds—Jim from plant pathology and me from soil microbiology—we both ended up working on soil health, cereal root diseases and conservation farming. Over a period of 25 years, Dr Glynn Bowen and I worked in parallel fields in CSIRO Division of Soils, which brought us together generating new ideas and experiments that resulted in many joint publications and culminated in a wide-ranging review of the topic (Bowen and Rovira 1999). This has been a relationship that I very much appreciate and one that contributed greatly to my research. Subsequent reviews by Garbeva *et al.* (2004), Bais *et al.* (2006) and Watt *et al.* (2006) have different emphases from our review.

After some years of fundamental research I decided to make the research more applied and extended the definition of the rhizosphere to include studies on plant growth promoting bacteria, soil-borne root pathogens, biological control of root diseases, the development of suppression to root pathogens in the field and the impact of rotations and tillage on root diseases, plant yields, water use efficiency and ultimately profitability when put into farming practice.

There is an enormous amount of published information on the rhizosphere (Lynch 1990) and with the concentration on recent information in computer literature searches, much of what I discuss will not be known to current scientists. However, I trust that this personal journey will interest you and inspire you to continue your research on the rhizosphere.

Techniques and stepping stones

Progress in science depends upon the developments of new techniques, often in unrelated fields, and the rhizosphere is no exception. I see these techniques as stepping stones towards greater knowledge of the subject and will now list some of these.

Light microscopy of root surface

Rovira (1956a), using direct microscopy with appropriate staining of lightly washed roots, provided some of the first information on the variety of organisms and their distribution on the surfaces of different parts of the roots. Louw and Webley (1959) reported that plate count estimates of rhizosphere bacteria were 30% of the direct microscope counts, indicating that many bacteria living on roots would not grow on the media used. Later studies by Rovira *et al.* (1974) using direct microscopy of lightly washed roots of eight pasture species showed that bacteria covered some 7.7% of the root surface, and that for these plants plate counts were only 10% of the total (direct) counts.

Nutritional groupings of rhizosphere and soil bacteria

Lochhead and his co-workers in Canada clearly demonstrated that those organisms stimulated by plant roots were generally faster growing and had different nutritional requirements from the bacteria in the soil away from the roots (Katznelson *et al.* 1948).

Selective techniques for different groups of bacteria

Work by Sands and Rovira (1971) and Simon *et al.* (1973), using selective media, found that fluorescent pseudomonads were associated with roots and freshly added particulate organic matter in soil. Subsequent research has found that many of these pseudomonads antagonise root pathogens and have the ability to promote plant growth. *Bacillus* species can be isolated by heat treating soil suspensions and many such isolates have been found to be active antagonists against root diseases, capable of promoting plant growth, increasing phosphate uptake and in inducing disease resistance in plants.

Paper chromatography

The first identification of amino acids and sugars in root exudates came in the 1950s by growing peas and oats in nutrient solutions and using paper chromatography developed by Swedish scientists for biochemical studies. For the first time we had information on the wide range of soluble organic compounds coming from healthy roots and providing sustenance for the rhizosphere microflora (Rovira 1956b).

^{14}Carbon labelling of plants

McDougall (1965), working in CSIRO, was one of the first to use C-14 labelling to study root exudation. Later, McDougall and Rovira (1970) used C-14 labelling of plants to investigate the sites of exudation from roots, while Rovira and Ridge (1973) used this technique to study the influence of plant nutrition on exudation. Whipps (1990) demonstrated that sloughed-off root cells from healthy plants contributed more carbon to the rhizosphere than did soluble exudates—he coined the term 'rhizodeposition' to describe the non-soluble carbon coming from healthy roots.

^{32}Phosphorus labelling of plants

Bowen and Rovira (1966) used this technique with solution-grown tomato and subterranean clover to demonstrate that non-sterile roots took up twice as much phosphate as sterile roots and during the course of the experiment translocated two to four times more phosphate to the tops than did sterile plants. Further studies showed that although microbial populations were very low in the root-tip region with heavy colonisation in the older part of the root, the apical region of non-sterile roots had a higher phosphate uptake compared with sterile roots, indicating that the micro-organisms on the older part of the root affected the metabolism of the root tip (Bowen and Rovira 1969).

Transmission electron microscopy of roots

The application of electron microscopy to roots grown in soil has provided us with an insight into the complexity of the rhizosphere (Foster *et al.* 1983) and of the many different types of bacteria living side by side within 10–20 microns of the root surface (Fig. 1).

Scanning electron microscopy (SEM) of the rhizosphere

This technique has the advantage that it gives a 'birds eye' view of the inner rhizosphere and the root surface. SEM examination of roots infected with the take-all fungus (*Gaeumannomyces graminis*) showed that the infection sites become heavily colonised by bacteria, and if these are effective biocontrol agents the technique showed that these bacteria lyse the hyphae of the pathogen (Fig. 2).

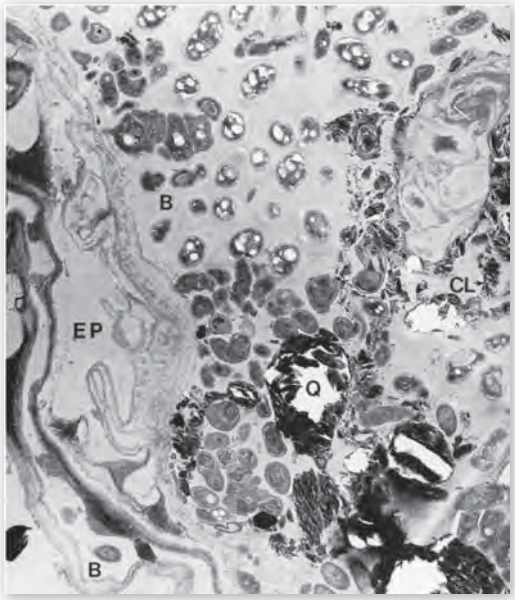

Figure 1. Rhizosphere of clover from the surface of an epidermal cell out to 10 microns from the root surface; B = bacteria, Q = quartz grain, CL = clay, EP = epidermal cells

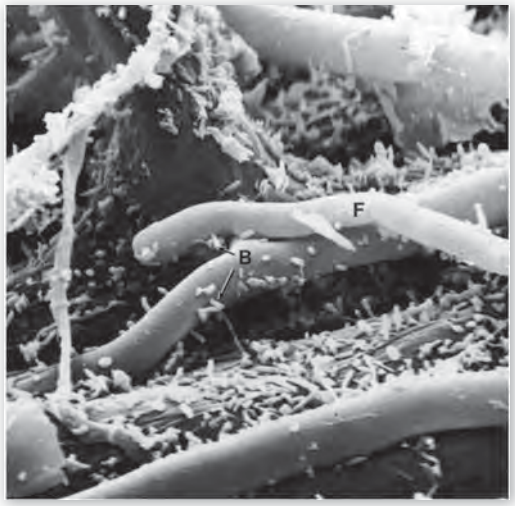

Figure 2. Surface of a wheat root infected by *Gaeumannomyces graminis* var. *tritici* showing hyphae of the pathogen (F) and a biocontrol bacteria (B) colonising both the root surface and the hyphae. Courtesy of R. Campbell, University of Bristol, Foster *et al.* (1983).

DNA detection of plant root pathogens in soil

The development of DNA assays for a number of cereal and pasture root pathogens by McKay and Ophel-Keller in SARDI (The South Australian Research and Development Institute) has led to the development of a Rood Disease Testing Service for farmers in southern Australia. Over the last ten years thousands of soil samples have been tested for root pathogens including *Rhizoctonia solani*, *Gaeumannomyces graminis* (take-all disease of cereals) and *Heterodora avenae* (cereal cyst nematode). The results are delivered to farmers via advisers who are trained to interpret the results. Each year McKay and associates have drawn maps showing the distribution and levels of pathogens in cereal-growing soils across southern Australia (Ophel-Keller *et al.* 2007). More recently this technique has been extended to root diseases of pasture legumes in southern Australia (I. Riley, SARDI, 2007, pers. comm.) and studying root distribution of crops and pastures (McKay *et al.* 2008).

This work of McKay, Ophel-Keller and associates is an outstanding example of how a new technique developed in unrelated fields can be applied to soil biology.

Root disease decline, suppression and biological control

This topic has interested plant pathologists for many years. The book by Cook and Baker (1983) is still the best treatise on the subject even though it was published 25 years ago. Take-all decline, where cropping continuously with wheat first leads to high levels of take-all disease and then leads to a decline, has been reported from the USA, UK, The Netherlands, Yugoslavia and Australia (Hornby 1979; Cook 2007a). Cook and Rovira (1976) demonstrated that it was possible to transfer the suppressive property by adding 1% of suppressive soil from a continuously-cropped wheat field to fumigated soil. Addition of soil from an adjacent uncropped area did not confer suppression to fumigated soil. Studies by Weller *et al.* (2002, 2007) and de Souza *et al.* (2003) have shown that fluorescent pseudomonads producing the antibiotic 2,4-diacetylfluoroglucinol (2,4-DAPG) occur in large numbers in the rhizosphere of wheat growing in take-all decline soils in the USA and in the Netherlands. These workers have extracted 2,4-DAPG from the rhizosphere of wheat growing in suppressive soils at levels high enough to inhibit the take-all fungus. Rotenberg *et al.* (2007) found that rotation and tillage affected the levels of 2,4-DAPG-producing pseudomonads in the rhizosphere, but the presence of pseudomonads was not always linked with disease suppression.

My opinion is that although Weller and associates have shown the links between these pseudomonads and take-all suppression, there will be other organisms and other antibiotics involved in suppression in other situations.

Simon *et al.* (1988), working in Western Australia, found that take-all suppression associated with prolonged use of nitrogenous fertilisers and continuous wheat cropping was due to the build-up of *Trichoderma koningii*. Subsequently Simon (1989) reported that the introduction of *T. koningii* (Strain Tk7a) into soil controlled take-all of wheat in controlled-environment conditions. This work has since been extended to the field in China and Australia with considerable success. In reviews by Vinale *et al.* (2006, 2008) many different mechanisms by which trichoderma inhibits root pathogens are described.

Another example of a different antifungal metabolite from a biocontrol fluorescent pseudomonad (*Ps.* str. AN5) is provided by Nayudu *et al.* (2010), who found that the active antifungal compound is gluconic acid, a simple hydrophilic sugar acid that is very effective against the take-all fungus.

Long-term rotation and tillage trials in South Australia have demonstrated that suppression can be built up against rhizoctonia root rot (Fig. 3) and take-all (Fig. 4) after the diseases peaked. This suppression developed in all rotations and in both direct drilled crops and crops sown after cultivation provided the crop residues were retained. This suppression was affected by the carbon-to-nitrogen ratio of the residues being returned to the soil. Suppression was maintained as long as the residues had a high C:N ratio (Roget and Gupta 2006). The incidence of rhizoctonia root rot has been difficult to predict and did not appear to be affected by rainfall. By contrast, in southern Australia take-all was affected by the rainfall in the previous spring and until suppression occurred it was possible to predict the potential level of take-all based on the previous year's rainfall, as shown in Figure 4 in those rotations that hosted the take-all fungus.

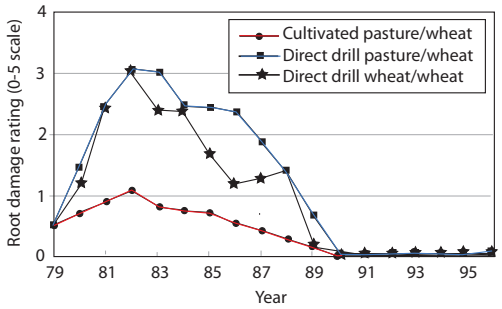

Figure 3. Suppression of rhizoctonia root rot with different rotations and tillage systems at Avon, South Australia (Roget 1995)

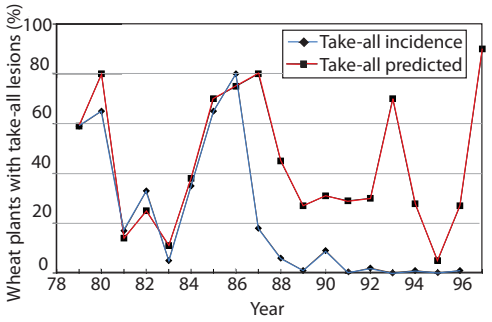

Figure 4. Development of suppression of take-all at Avon, South Australia. This graph shows the actual levels of take-all and the predicted levels based on rainfall (Roget 2003)

Table 1. Numbers of bacteria[a] in soil and on wheat straw from burnt and unburnt areas of stubble at Turretfield, South Australia

Treatment	Viable count / g on wheat stubble			
	Straw retained		Straw burnt	
	FP[b]	TB[c]	FP	TB
Soil	25	1.8	14	4
Straw				
Slight decomposition	10 300	60	240	21
Advanced decomposition	316	319	420	252

[a] Numbers are means of duplicate or triplicate samples
[b] Fluorescent pseudomonads ($\times 10^3$/g)
[c] Total bacterial count on YPS agar ($\times 10^6$/g)

This effect of crop residues may be explained in part by the finding of Rovira and Sands (1971) some 35 years earlier that under stubble there were almost 50 times more fluorescent pseudomonads in the soil than were found where the stubble had been burned (Table 1).

Another example of root disease decline came from Kerry and Crump (1977), who found that cereal cyst nematode (*Heterodera avenae*) (CCN) built up and then declined with continuous cereal cropping. This decline was attributed to biological control by two fungi, *Nematophthora gynophyla* and *Verticillium chlamydosporium*. Following this report, CSIRO invited Brian Kerry to visit Adelaide to investigate the potential for biological control of CCN as it was a major problem throughout south-eastern Australia. Stirling and Kerry (1983) examined more than 23 000 CCN females from 375 sites but did not find *N. gynophila*, but they did find small populations of the other parasite, *V. chlamydosporium*. Kerry *et al.* (1984) then explored the potential for *V. chlamydoporium* to control CCN by introducing the fungus grown on oat grain into soil containing CCN and then growing wheat in these soils. In these greenhouse experiments they found that CCN numbers were reduced by between 26 and 80% when the fungus was introduced. This study was not extended to the field because chemical control of CCN and rotation were being used to effectively control CCN. Soon after this work plant breeders bred resistant cereals, making both biological and chemical control obsolete. It is unlikely, however, that biological control was significant in Australian soils because CCN never showed any signs of declining unless non-host plants were grown in rotation with cereals.

Biological control of root diseases by seed or soil treatment

Since the discovery that many soil micro-organisms produce antifungal and antibacterial compounds there have been many attempts to control root diseases by introducing such organisms either by seed or soil treatment. Although many hundreds of potential biocontrol organisms have been tested and found to be effective in pot and field trials, only 14 bacteria and 12 fungi were registered by the US EPA for use in agriculture and horticulture (Fravel 2005). One reason for this may be the high cost of field testing and marketing of potential biocontrol agents, while another may be that biocontrol agents may be more affected by adverse soil conditions than are chemical agents.

This indicates the difficulty of controlling root diseases by seed or soil treatment, so much so that Cook (2007b) and Mazzola (2007) postulated that cropping systems that increase the populations of antagonists in soil and in the rhizosphere offer a preferable route.

Despite this view, however, I still believe that as our knowledge of rhizosphere dynamics increases we will be able to use the inoculation approach. After all, the symbiosis of the nitrogen-fixing *Rhizobium* with legumes following seed treatment with rhizobium makes an enormous contribution to food production world wide (Crews and Peoples 2004). In Australia this symbiosis is estimated to be worth AUD$3 billion each year to agricultural production, based on the cost of its replacement with fertiliser nitrogen (Howieson and Herridge 2005).

Two major factors mitigate against the success of seed or soil treatment to control root diseases. The first is to achieve colonisation along the root, and this was shown to be difficult with a biocontrol pseudomonad without having water movement down the profile (Parke *et al.* 1986). The second is the complexity of the rhizosphere, as shown by electron microscopy of roots (Fig. 1) that indicates the environment into which we are introducing beneficial organisms. However, the fact that there have been successes in several countries as described below is cause for optimism.

In China, the use of *Bacillus* spp. in the commercial YIBS (Yield-Increasing Bacteria) product and the use of *Trichoderma* spp. and other organisms demonstrates the potential of seed and soil treatment with beneficial organisms (Tang and Rovira 2005).

In Australia there are some examples of root disease control and yield increases from seed or soil treatment with biological control agents. Nayudu *et al.* (2010) has obtained yield increases ranging from 10 to 32% from planting seed treated with a fluorescent pseudomonas into fields infested with the take-all fungus in southern New South Wales, which has a higher rainfall than other wheat-growing areas of Australia. Ryder *et al.* (2005) has obtained similar though variable responses from seed treated with a fluorescent pseudomonad and with in-furrow treatment with *Trichoderma koningii* (strain Tk7a from Western Australia). Wakelin *et al.* (2006) found that a combination of *Penicillium radicum* and the fungicide fluquinconazole gave better control of take-all than either the biological treatment or the fungicide alone.

Coombs *et al.* (2004), using several endophytic actinobacteria, found that some of the isolates greatly reduced damage from the take-all fungus and from rhizoctonia in the field. These endophytic actinobacteria have an advantage over other biocontrol agents in that they invade the wheat roots soon after germination and thus do not have to compete with the general rhizosphere population.

In a large-scale field study, Putcha and Allen (1997) applied the liquid suspensions of 600 isolates with cotton seed in the drill rows and reported that several of the isolates were equal to or superior to chemicals in controlling cotton seedling diseases.

Impact of root diseases, rotation and tillage on root disease and yield

The effect of cropping systems on rhizosphere organisms that affect plant health is illustrated in the review by Rovira *et al.* (1990). In my research program on root health and yield I used soil fumigation and rotations to demonstrate the impact of root diseases on grain yield and water-use efficiency. One of these trials conducted in Victoria, using a nematicide to control cereal cyst nematode, fumigation to control all root pathogens and nitrogen fertiliser, showed spectacular yield increases from controlling root diseases (Fig. 5; Meagher *et al.* 1978).

Fumigation of highly calcareous soils in South Australia gave similar increases in grain yield, which was attributed to the control of a root disease complex (Rovira and Simon 1985).

These soil fumigation trials led to a long-term tillage × rotation trial at Avon, South Australia, where take-all and rhizoctonia root rot were problems.

This was the site where suppression to rhizoctonia appeared after four years and suppression to take-all appeared after seven years (see Figs 3 and 4). Figure 6 shows that control of root diseases by rotation and tillage before suppression occurred was necessary to maximise yield and maximise water-use efficiency (WUE).

Figure 7 demonstrates that unless take-all is controlled by rotation, yields from direct-drilled wheat do not reach the yields of wheat planted with cultivation.

Subsequent research at this site led to the development of narrow sowing points for direct drilling — these points disturbed the soil below the seed, controlling rhizoctonia sufficiently for the seedling roots to escape the disease (Roget *et al.* 1996).

Pythium spp. is a root pathogen with a wide host range and often associated with cold, wet conditions at sowing, causing yield losses of 5–10% (Ingram *et al.* 1990). In South Australia, Pankhurst and McDonald (1988) reported higher levels of *Pythium* following the annual pasture phase of a rotation than after the cereal phase. Clive Pankhurst (CSIRO, pers. comm.) demonstrated that infection of wheat seed embryos occurred within 48 hours of planting, which means that there is a good opportunity to control this disease with seed dressings of chemicals or biological control agents.

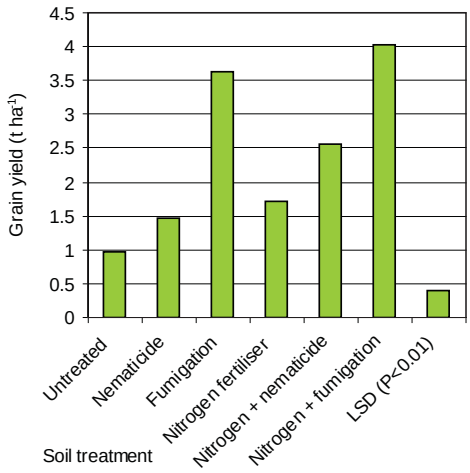

Figure 5. The effects of soil fumigation with methyl bromide-chloropicrin, nematicide (aldicarb) and nitrogen fertiliser on grain yield (Meagher *et al.* 1978)

They found that the most profitable system was continuous cropping, with wheat alternating with peas both sown directly into stubble with no nitrogen fertiliser (Fig. 8).

This rotation kept the level of take-all down and benefited from the nitrogen fixed by the peas. The rotation which gave the second-best financial return was wheat alternating with oats (not a host for the take-all fungus) planted with cultivation.

Economic value of farming systems research

If we wish to make our rhizosphere studies more relevant to real farming, an economic analysis must take into account all inputs for each treatment (seed, fertiliser, chemicals, fuel) with the different farming systems. David Roget, together with Mike Krause (an agricultural economist), applied a rigorous economic analysis to the Avon trial.

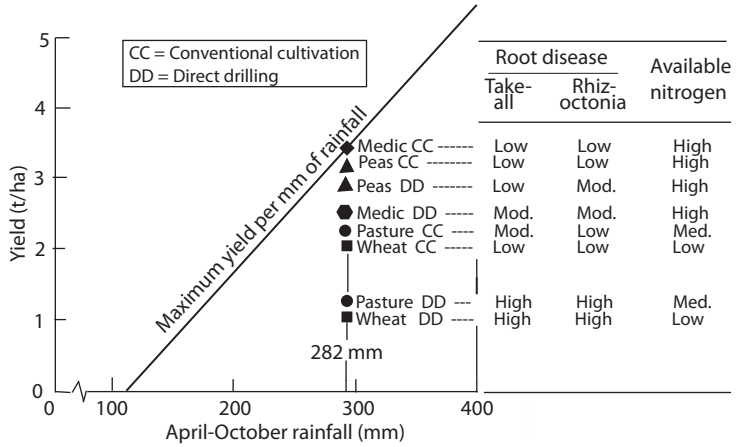

Figure 6. Impact of root disease and soil nitrogen on grain yield and water use efficiency

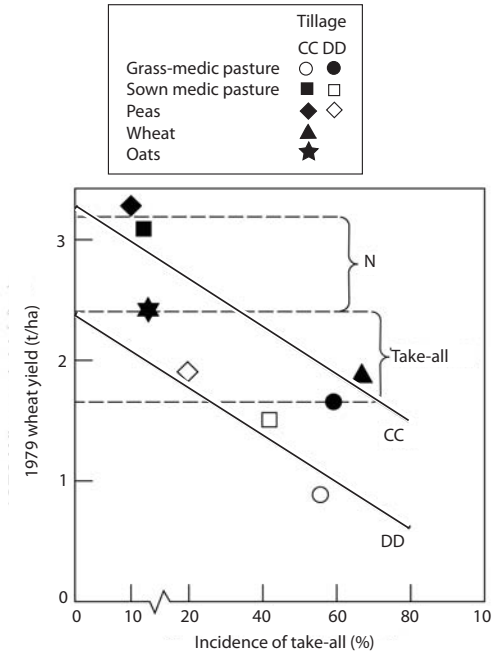

Figure 7. Effect of rotation and tillage on the incidence of take-all disease and grain yield of wheat during 1979 at Avon, South Australia. Values are averages of four replicate plots (Rovira, unpublished). DD = direct drilling, no cultivation before seeding; CC = conventional cultivation before seeding. Regression values (r^2) for CC and DD were 0.79 and 0.91 respectively.

Since these trials were completed and analysed there has been widespread acceptance in southern Australia of growing successive wheat crops with direct drilling and stubble retention.

Importance of the rhizosphere microflora on successive crops

Following the wider adoption of continuous cropping with wheat in Australia it has been found that yields of wheat in crops following certain other varieties of wheat were 5–20% lower than expected. Gupta *et al.* (2004) demonstrated that this reduced yield was due to the rhizosphere microflora on the roots of the wheat variety causing lower yields in the following wheat crop.

Mazzola *et al.* (2004) demonstrated that certain wheat cultivars selectively stimulated fluorescent pseudomonads that produced the antibiotic 2,4-diacetylphloroglucinol from resident soil populations.

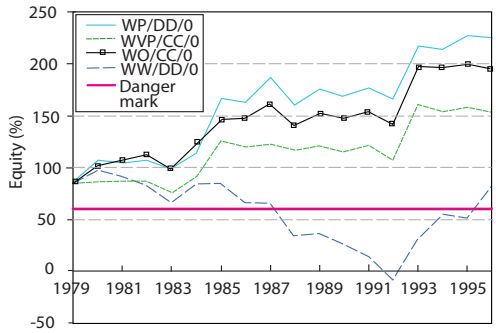

Figure 8. The profitability of the different rotations and tillage treatments expressed as change in equity in a long-term farming system trial at Avon, South Australia. WP = wheat/peas; WVP = wheat/volunteer pasture; WO = wheat/oats; WW = wheat/wheat; DD = direct drill; CC = conventional cultivation; 0 = zero fertiliser. 'Danger mark' refers to an equity point where the business is reaching a non-viable condition (David Roget and Mike Krause, 2000, pers. comm.)

This property means that these cultivars may have a greater 'resistance' to the take-all fungus and the use of such cultivars in wheat breeding programs may provide an indirect control of the disease in successive wheat crops.

These reports by Gupta *et al.* (2004) and Mazzola *et al.* (2004) present a challenge to plant breeders to gain a better understanding of the importance of the rhizosphere microflora to reduce the impact of one variety upon another and in the resistance to root diseases.

Plant growth – promoting rhizobacteria (PGPR)

Research conducted some 20 years later by Dobereiner *et al.* (1976) reported that *Azospirillum* fixed nitrogen in the rhizosphere of paspalum (a tropical grass) in Brazil. These reports caused considerable excitement in the USA where *Azospirillum* treatment of crops such as corn was seen as an alternative to the use of nitrogenous fertiliser. Unfortunately, there was no recognition of the research conducted in Russia, England and Australia with *Azotobacter* that showed that plant growth responses were not due to nitrogen fixation, and it was not until a great deal of research was conducted across the USA with variable results that it was con-

cluded that inoculation with *Azospirillum* did not provide a substitute for fertiliser nitrogen (Bashan and Holguin 1997). I think that the researchers failed to appreciate the very different soil environment under a paspalum pasture (see Figs 50–53 in Foster *et al.* 1983) compared with the rhizosphere of an annual cereal such as corn (maize). Under paspalum and around the paspalum roots there is a very high level of available substrate for the *Azospirillum* that is conducive to nitrogen fixation. The higher soil temperature and high rainfall of tropical Brazil would also be conducive to microbial activity, including non-symbiotic nitrogen fixation.

Studies by later researchers showed that many bacteria promoted plant growth, and some of these results have been reported in the series of PGPR (plant growth-promoting rhizobacteria) workshops held over the past 20 years. The proceedings of each of these PGPR workshops provide an excellent source of information on the effects of rhizosphere microbes on plant growth.

Induced resistance to root and foliar diseases

Kloepper *et al.* (2004, 2006) have shown that many PGPR pseudomonads and *Bacillus* spp. can induce resistance to a wide range of foliar diseases of plants, and in some cases increase resistance to insect pests. Most of these PGPR organisms colonise the rhizosphere, but some are endophytes which could give them an advantage through avoiding competition.

Impact of cruciferous plants on root disease

For many years in India mustard has been planted between rows of other crops to control fusarium wilt in the crop plants, an effect attributed to volatile compounds being released from the mustard roots. Studies by Kirkegaard and Sarwar (1998) reported less take-all in wheat crops following canola and mustard, and attributed this to the release of isothiocyanates from roots or residues and their conversion to glucosinolates resulting in 'biofumigation' of the soil. However, the relative importance of the biofumigation effects of canola compared with its value purely as a non-host crop for the take-all fungus is questioned in a later paper by Smith *et al.* (2004).

Mycorrhizas and crop yields

In the Darling Downs of southern Queensland a condition known as 'long fallow disorder' caused poor growth of wheat following a period of bare fallow. This was attributed to a nutrient disorder as it could be partly overcome with applications of zinc and phosphorus. However, Thompson (1994) demonstrated that during the fallow period the levels of mycorrhizal fungi declined to the point that the following wheat plants were not mycorrhizal, and in the high-clay soils the roots alone could not access the soil phosphorus. Thompson showed that if the fallow period was shortened, or crops which became mycorrhizal were grown before the wheat, the problem was overcome. It is more difficult to show the importance of mycorrhizas in crops in other soils, but Smith and co-workers (Sally Smith, University of Adelaide, 2008, pers. comm.) have shown that introduction of mycorrhizal fungi at sowing of wheat in highly calcareous, phosphate-fixing soil increased the uptake of phosphorus.

Plant nutrition and root health

Early studies reported by Rovira and Ridge (1973) that plant nutrition affected the exudation of soluble compounds from plant roots has relevance to the findings of Wilhelm *et al.* (1988) and Graham and Rovira (1984) that the application of manganese fertiliser to manganese-deficient soils reduced the colonisation and invasion of roots by the take-all fungus (*Gaeumannomyces graminis* var. *tritici*) and increased grain yields because of less disease. Another example of the effect of nutrition on root disease was reported by Thongbai *et al.* (1993) who found that application of zinc to zinc-deficient sandy soils greatly reduced the severity of rhizoctonia root rot.

Future prospects for rhizosphere research

I believe that the development of new and recently developed techniques applied to rhizosphere studies will enable us to ultimately modify the rhizosphere and improve plant production. It is my opinion that we need to include root pathogens in our definition of the rhizosphere as they can have a devastating impact on food production. Research needs to progress at two levels—firstly, fundamental studies that improve our understanding of the dynamics

of the rhizosphere and secondly the application of these results to field problems. Below are some areas of research which I believe will be important in the future.

DNA quantification of pathogens, introduced organisms and roots

Over the past ten years, this technique has yielded a great deal of information on the levels and distribution of cereal root pathogens in southern Australia. This technique could be extended to follow the colonisation of roots by pathogens and introduced microbes. Possibly, it could be used as a technique to screen cereal varieties for the colonisation of roots by pathogens such as take-all, fusarium and rhizoctonia.

New techniques for culturing bacteria from the rhizosphere

The development of media to culture slow-growing soil bacteria (Janssen *et al*. 2002) has led to the discovery of many new species. This technique could be applied to the rhizosphere to improve our knowledge of the bacteria growing on roots. The use of culture-independent techniques such as genomics and microarrays will increase understanding of the nature of rhizosphere populations (Kent and Triplett 2002)

Use rhizosphere colonisation of roots to introduce beneficial organisms into soil

Microbial degradation of pollutants in soil is one method of decontaminating soils and it should be possible to use roots of plants such as grasses to distribute beneficial organisms through soil.

Improve rhizobium–legume associations to increase productivity

This symbiotic association has brought enormous benefits to agriculture world wide, but more knowledge is required to introduce rhizobia into hostile soils. The work of Dilworth *et al.* (2001) demonstrating how to select acid tolerance in legume root nodule bacteria, and the research by Howieson and Ewing (1986) using acid-tolerant selections of *Medicago* spp., has extended the range of soils in Western Australia in which *Medicago* spp. can be grown for annual pasture.

Develop mycorrhizal associations to increase nutrient uptake

In many soils phosphorus levels may be high but relatively unavailable to plants, so the use of mycorrhizas could be an advantage.

Develop methods of introducing beneficial organism and broaden target crops

It is now clearly established that there are many bacteria, actinomycetes and fungi that can improve plant production by controlling root disease, inducing resistance to pathogens and pests and increasing nutrient uptake. This is a difficult area to research, but nevertheless methods of producing and applying these bioinoculants should be developed so that food production world wide can be increased and the use of chemical controls reduced. So far, most research has been conducted on rain-fed broad-acre crops, but I believe that there is a huge potential to increase the production of irrigated vegetable crops by introducing organisms that will promote plant growth and control root diseases. Organisms could be introduced to the roots through trickle irrigation systems used for many vegetable crops.

Breeding crop varieties with resistance to soil-borne root diseases

The breeding of varieties of wheat, barley and oats with resistance to cereal cyst nematode (CCN) is an example how this particular root disease can be controlled to the point that yield losses in Australia are now negligible compared with 25 years ago, when the disease was causing yield losses of $40–$80 million each year (Rovira and Simon 1982).

Despite the findings that rhizoctonia root rot can be managed in many situations through suppression and sowing point design, it still causes heavy losses in calcareous sandy soils (Wilhelm 2010, pp. 55–60 this volume), so we need to consider breeding for resistance to this and other cereal root diseases.

A number of papers have reported tolerance and or resistance in wheat to crown rot (Dodman *et al*. 1985; Klein *et al*. 1985), take-all (Simon and Rovira 1985) and rhizoctonia root rot (McDonald and Rovira 1985), which should encourage cereal breeders to pursue breeding as a strategy to reduce yield losses from these diseases.

The discovery at Washington State University, Pullman, USA, of a wheat genotype with tolerance to rhizoctonia root rot (Okubara *et al.* 2008) should encourage cereal breeders in Australia to develop a screening program for tolerance or resistance to rhizoctonia root rot within the Australian cereal varieties.

You *et al.* (2005) have reported new sources of resistance in subterranean clover to *Fusarium avenaceum* and *Pythium irregulare* that should be valuable in increasing annual pasture production in southern Australia.

Conclusions

I hope that this personal journey has given you an insight on how my interests in rhizosphere biology developed from a chance reading of some reviews over 50 years ago to the point where, over the years, my interests have expanded from the basic aspects of the rhizosphere to the application of the knowledge to agriculture to develop more productive and sustainable farming systems. My definition of the rhizosphere is much broader than that used by most soil microbiologists and includes root pathology and plant breeding, but if we wish to use our understanding of the rhizosphere to increase food production I believe that we are obliged to use this broader definition.

The ATSE Crawford Fund which sponsored this symposium has the goal of reducing poverty in lesser-developed countries through increased food production. There is no doubt in my mind that a better understanding of the biology of the rhizosphere and application of that knowledge to field problems will help achieve that goal.

I thank the ATSE Crawford Fund and the organisers of this symposium for giving me the opportunity to prepare this personal journey through the rhizosphere.

Finally I wish to acknowledge the help given to me by Dr Gupta Vadakattu in coping with the vagaries of different computer programs while this manuscript was being prepared.

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Program for the Rovira Rhizosphere Symposium on 15 August 2008

Start	Finish	Speaker	Organisation	Title
0900	0930	REGISTRATION		
Sesson 1—John Radcliffe				
0935	0940	Neil Andrew	ATSE Crawford Fund	Opening remarks
0945	1015	Richard Burns	University of Queensland	Albert Rovira and half a century of rhizosphere research: are we any the wiser?
1015	1030	Gupta V.V.S.R.	CSIRO Entomology	How best can we design rhizosphere plant–microbe interactions for the benefit of plant growth?
1030	1045	Sina Adl	Dalhousie University	Trophic cascades in pine seedling rhizosphere caused by fungivory on establishing mycorrhizae
1045	1115	COFFEE BREAK		
Session 2 —Paul Harvey				
1115	1145	Jim Tiedje	Michigan State University	New technologies for more in-depth understanding of the soil and rhizosphere communities
1145	1200	Murali Nayudu	Australian National University	Nature of biocontol of take-all by the Australian bacterial isolate *Pseudomonas* strain AN5
1200	1215	Chris Franco	Flinders University	Co-opting the endorhizosphere
1215	1230	Sally Smith	University of Adelaide	New insights into roles of arbuscular mycorrhizas in crops and natural ecosystems
1230	1330	LUNCH		
Session 3—Kathy Ophel-Keller				
1330	1400	Dick Smiley	Oregon State University	Root diseases, plant health and farming systems
1400	1420	Neil Smith	Farmer, York Peninsula, SA	A farmer's perspective of the Rovira contribution to Australian agriculture
1420	1435	Nigel Wilhelm	SARDI	Soilborne diseases in southern Australian agriculture
1435	1450	Graham Stirling	Queensland	Minimum tillage and residue retention enhance suppression of *Pratylenchus* and other plant-parasitic nematodes in both grain and sugarcane farming systems
1450	1510	TEA BREAK		
Session 4—John Radcliffe				
1510	1530	Maarten Ryder, Tang Wenhua	Adelaide/China Agricultural University	Long-term international research cooperation between China and Australia on soil biology in agriculture
1530	1600	Hugh Wallwork, JulieNicol	SARDI /CIMMYT	ATSE Crawford International Masterclasses on soilborne pathogens of cereals
1600	1630	Albert Rovira	Adelaide	The biology of the rhizosphere: past, present, future
1630	CLOSE			

Poster presentations

Presenter	Organisation	Title
Rowena Davey	SARDI	Influence of different carbon sources in soil on populations of specific organisms linked to soil-borne disease suppression of *Rhizoctonia* root rot
Oliver Knox	Scotland	Varietal differences in cotton belowground
N.E. Reddy	India	Variability among the isolates of *Sclerotium rolfsii* (Sacc.) causing stem rot of peanut (*Arachis hypogaea* L.)
Richard Simpson	CSIRO	Root damage constrains autumn-winter pasture yield and farm productivity
Richard Winder	Canada	Development of a rnicrobial indicator database for validating measures of sustainable forest soils

Crawford Fund Publications since 2006

Most of these publications are available on the fund's website, http://www.crawfordfund.org

Brown, A.G. (ed.) 2006. *Forests, Wood and Livelihoods: Finding a Future for All.* Record of a conference conducted by the ATSE Crawford Fund, Parliament House, Canberra, 16 August 2005. The ATSE Crawford Fund, Parkville, Vic. vi + 91 pp. ISBN 1 875618 86 4

Anon. 2006. *The ATSE Crawford Fund Report 1 July 2005 – 30 June 2006*. The Fund, Parkville, Vic. 28 pp. http://www.crawfordfund.org/publications/pdf/annualreport2006.pdf.

Brown, A.G. (ed.) 2007. *Water for Irrigated Agriculture and the Environment: Finding a Flow for All*. Record of a conference conducted by the ATSE Crawford Fund, Parliament House, Canberra, 16 August 2006. The ATSE Crawford Fund, Parkville, Vic. vi + 72 pp. ISBN 1 875618 92 9

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Persley, G.J. and Blight, D.G. (eds) 2008. *A Food Secure World: How Australia can Help*. Report of the Crawford Fund World Food Crisis Task Force, Australian Academy of Technological Sciences and Engineering (ATSE), Melbourne, 60 pp. ISBN 978-1-921388-00-2

Anon. 2008. *The Crawford Fund: An Initiative of the Australian Academy of Technological Sciences and Engineering. Annual Report 1 July 2007 to 30 June 2008*. The Fund, Deakin, ACT, 36 pp. http://www.crawfordfund.org/publications/pdf/annualreport2008.pdf

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Duncan, R., Tait, R. and Keating, B. 2009. *Independent Review of the Crawford Fund*. The Crawford Fund, Deakin. 70 pp.

Brown, A.G. (ed.) 2010. *World Food Security: Can Private Sector R&D Feed the Poor?* The Crawford Fund Fifteenth Annual Development Conference, Parliament House, Canberra, 27–28 October 2009. The Crawford Fund, Deakin, ACT. viii + 116 pp. ISBN 978 1 921388 08 8

The Crawford Fund newsletter, *Highlights*, is available from the Fund's website or in printed form. The most recent issue was published in April 2010.

The three publications below discuss the global setting for international agricultural research. The website of the Cooperative Group for International Agricultural Research (CGIAR) (http://www.cgiar.org/) provides other information.

Alston, J.M., Pardey, P.G. and Taylor, M.J. (eds) 2001. *Agricultural Science Policy: Changing Global Agendas*. John Hopkins University Press, Baltimore, 285 pp. ISBN 0 8018 6603 0

Pardey, P.G., Alston, J.M. and Piggott, R.R. (eds) 2006. *Agricultural R&D in the Developing World: Too Little, Too Late?* International Food Policy Research Institute, Washington DC. Available for download from http://www.ifpri.org/pubs/books/oc51.asp

World Bank 2007. *World Development Report 2008: Agriculture for Development*. World Bank, Washington, D.C. xviii + 365 pp. http://econ.worldbank.org ISBN: 9780821368077

The Crawford Fund facilitated an award-winning one-hour TV documentary, *Seed Hunter* (1988), that follows Australian scientist Dr Ken Street on a quest through Central Asia to find rare genes that may save our food from the looming threat of climate change. More details are at http://www.seedhunter.com/.