final report

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Pasture Soil Biology 2003-2008

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Preface

This report summarises information submitted in project reports by contractors engaged in the Program and largely represents the information provided in executive summaries. On occasion, the author of this report has added brief comments.

Full reports from Program projects may be obtained on application to MLA.

Recommendations and suggestions for future research in soil biology are those of the author although taking into account ideas raised by participants in the soil biology program and others.

Abstract

Phase 1 of the Pasture Soil Biology Program has been successfully completed with evidence that soil biological constraints to pasture productivity are significant and widespread which represents a significant opportunity cost in foregone livestock production and carbon storage in plant material for soil health improvement.

The program has successfully developed a wide range of new molecular assays that can identify and quantify targeted soil borne micro-organisms, both pathogens and beneficial organisms, important to pasture plant productivity. A breakthrough innovation has been to develop molecular assays for selected pasture plants which can be applied to study the dynamics of root growth and response to external influences. These assays leave a significant legacy to researchers to help accelerate research into soil biology under pastures and in pasture-cropping systems.

Evaluation of molecular assays for soil biological function has confirmed that these assays are valuable research tools to study soil biological diversity and nutrient cycles with reference to pasture production and, importantly, the environmental foot print of pasture systems in terms on greenhouse gas emissions and nutrient leakage.

Basic science studies on soil biota in the plant rhizosphere are on-going and have suggested that chemical signalling between plant roots and soil borne organisms may lead to innovative ways to influence plant performance and responses to stresses such as soil borne pathogens.

A Program review, strongly endorsed the over-arching Program strategy and that it continue over a sufficient time frame to capitalise on the achievements so far to attain the aspirational target of at least 10% increase in pasture productivity.

Recommendations are made as a framework to continue this work such as to have parallel themes on production, adaptation to climate change and environment with special emphasis on understanding the dynamic interactions in pasture systems with a view to develop management practices for soil biological health.

A business case for continuing soil biology research has been developed.

Executive Summary

Program Development

The Pasture Soil Biology Program was established as an MLA Strategic Science initiative in 2003 to develop enabling technologies and knowledge in support of enhancing soil biological health under pasture systems if importance to the red meat industries for outcomes in improved productivity and sustainability. Evidence in the literature suggested that removal of soil biological constraints had supported dramatic increases in pasture dry matter production.

Based on a "Knowledge and Opportunity Audit" and other evidence from the literature and consultations, a Program strategy was developed with a long term vision within which a series of phases were conceptualised:

- Develop new research tools and knowledge to enhance the capacity to conduct research in soil biology relevant to pasture systems.
- Use these tools to investigate and understand the dynamic interactions between soil type, pasture type, season and management and essential elements of soil biology composition, function and impact on plants
- Use this knowledge to develop management practices for producers to enhance productivity and sustainability through improved soil biological health.

A feature of the strategy was to include both basic science and more applied projects linked to a common purpose to advance knowledge and capability in soil biological research.

The aspirational target was agreed for a 10% increase in pasture productivity which MLA modelling suggested would result in a grazing industry benefit of c. $260m per annum

An investor alliance was formed with AWI and GRDC joining as co-contributors. Participation of GRDC was sought to ensure strong links with the GRDC Soil Biology Initiative ($10m over 5 years) which was current at the time. A total investment of $2.65m was committed over four years.

Dr R Hannam (R J Hannam & Co Pty Ltd) was appointed as Program Coordinator and a series of projects established to commence the Program with emphasis on southern Australian pasture systems and objectives to:

- Develop molecular assays, as research tools, to identify and quantify specific soil borne organisms important to pasture soils
- Validate the evidence that soil biological constraints to pasture productivity are significant
- Investigate interactions in the rhizosphere of pasture plant roots and soil microbes

Program Achievements

During the period July 2003 to June 2008, the following has been achieved:

- Field bioassays have suggested that there is widespread root damage in sub-clover, ryegrass and Lucerne particularly in the early season autumn-winter period even in conditions not conducive to soil borne pathogens. Estimates are that the pastures investigated are producing to only c. 60% of their potential with a significant potential loss of seed and seedlings affecting pasture persistence and livestock production. This was confirmed in two series of experiments.

- A literature review confirmed that soil borne pathogens induce significant constraints to pastures in terms of reduced plant growth, seed set and viability, plant nutritional value and palatability, nitrogen fixation, input utilisation, poorer pasture composition, increased risk of mycotoxins and soil erosion plus increased costs of production.
- Molecular assays were successfully developed for a wide range of soil borne pathogens and beneficial organisms of significance to pastures as research tools. DNA assays have also been developed for selected plants which can be used to study the dynamics of root growth and response to environment, management and season. Collectively, these assays offer a powerful inventory of research tools to accelerate knowledge development of the dynamic interactions between plants and soil biota in pasture systems with a lot information gathered from single DNA samples.
- Molecular assays for soil biological function were also evaluated as tools to study soil microbial diversity and nutrient cycling and were confirmed as useful tools to study the environmental impact of contrasting pasture and management systems on factors such as greenhouse gas emissions.
- The molecular assays have been successfully applied to field sites in collaboration projects such as EverGraze, MASTER (long term lime trial) and a CSIRO PI sponsored long term P x grazing trial to confirm that they can identify and quantify treatment differences in soil borne organisms and soil biological function as will be valuable in investigating the biological dynamics in pasture soils.
- A highlight has been the assays for plant roots which detect live cells and offer the potential to revolutionise the capacity of scientists to study the dynamics and responsiveness of plant roots rather than relying on crude estimates of root mass
- Training programs were developed and run for researchers to introduced them to the new technology and encourage their use of the new research tools.
- The basic science studies on interactions between roots and microbes in the rhizosphere have established that bio-chemical signals (quorum sensing signals) offer an opportunity to influence plant responses to soil borne organisms.
- Research focussed on developing knowledge of how plant roots respond to stress and management by changing their rhizosphere environment and how this interacts with rhizosphere micro-organisms was commenced.
- A Program review strongly endorsed the over-arching strategy, the program management and achievements. It strongly recommended continuance. They confirmed that the aspirational production improvement target is achievable.

Industry Benefits

The foundation has begun to be set to accelerate the development of knowledge and capability to develop management tactics for enhanced soil biological health, improved pasture productivity and profitability from livestock systems. This is a long-term Program where industry benefits should be begin to be captured during the next 4-5 years if the work is continued.

Outcomes will be further enhanced by better managing the environmental impacts of grazed pasture systems and better adoption of successful strategies to adapt to climate change and variability.

The Future

Recommendations for future research in pasture soil biology are described which includes parallel but linked themes on:

- **Production** – continue to pursue the aspirational target of increasing pasture productivity by 10%
- **Adaptation – Climate Change** – characterisation and development of adaptation strategies management of greenhouse gas emissions from pasture systems, probably with the emphasis on nitrous oxide due to its very high potency and methane as well as aspects of soil carbon sequestration and transformations.

An expansion of the Program to northern Australian pasture systems to address issues specific those environments is also suggested.

Specific recommendations are:

- **Ecology & Management of Root Damage in Pastures**
    - Continue field bioassays across more sites using the DNA assays to identify causal organism and also help calibrate the level of pathogens that represent risk of plant yield loss. This will be important to developing the DNA assays and diagnostic and monitoring tools for producers in aiding management decisions.
- **Molecular Assays as Research Tools – Production**
    - Continue development and validation of assays for organisms and plant roots important in pasture and pasture/crop systems – correlate with factors affecting production and environmental impacts
    - Evaluate assays for general soil biology parameters which relate to soil health
    - Continue to educate and engage with researchers on their use in research and monitoring
- **Nitrogen and Carbon Cycles in Pasture Soils – Production and Environment**
    - Use a combination of molecular microbial, soil function and chemical analytical techniques to assess pasture management, pasture type, soil type and season on:
        - The N and C cycles with special emphasis on greenhouse gas emissions, systems transformations, leakage and storage.
        - Fungi and bacteria microbial community diversity and function
    - Link with the effort on production and interactions with other organisms.
    - Place special emphasis on micro-ecological studies of carbon turnover, microbial community analysis and soil health in relation to pasture systems and pasture-cropping systems. Objective to develop basis for soil health benchmarks.
- **Pasture Plant Pathologist – Soil Biologist**
    - Engage a early career pasture pathologist to develop skills in traditional and molecular aspects of soil pathogens and beneficial organisms in soil and also develop skills in general pasture soil biology
    - This position should offer a significant career prospect and should be seen as a key plank in developing research capacity in this area for then next 10-15 years.
- **Lucerne establishment and productivity – Mallee and contrasting areas**
    - Resolve the risks of establishing Lucerne phases in cropping systems which recent surveys have shown to be affected by soil borne pathogens affecting establishment, survival and productivity.
    - Place some emphasis on management to optimise crop-pasture-crop transitions in mixed farming systems.
- **Interactions – Plant Roots and Soil Microbes in the Rhizosphere**

    - Continue basic research into understanding interactions at the root surface with clearly defined researchable questions of importance to plant productivity, disease resistance/tolerance and interactions with beneficial soil organisms.
- **Quorum Sensing Signals (QSS)**
    - Continue basic science investigating the signalling between plant roots ad soil microbes with a view to identify systemic signalling chemicals which can benefit plant performance and resilience.
- **Mechanisms in plants of resistance and tolerance to soil borne pathogens**
    - Strategic basic science to underpin breeding programs for these traits and development of disease mitigation management practices.
    - Include interactions with mineral nutrition – two key elements that constrain root growth – pathogens and nutrients
- **Root Growth in Soils**
    - Use the molecular assays for plant roots to study how plant roots penetrate hostile soils
    - Link to plant improvement programs to evaluate emerging varieties for better root penetration and survival in these soils – feedback to breeders on important traits.
    - Link to research on adaptation to climate variability and change – successful root growth and function will be a critical success factor for adapted plants
- **Beneficial Endophytes to Protect Pasture Plants from Soil Borne Disease**
    - Identify systemic endophytes as seed treatments for pasture seed to confer early vigour and resistance/tolerance to soil borne pathogens
    - Link with the QSS research
- **Soil Ameliorants – Impacts on Soil Biota**
    - Evaluate conventional and unconventional soil ameliorants (eg lime, charcoal, “organic” lobby recommendations etc) on soil biota as measured with the molecular assays.
    - Engage with producer groups to validate claims of soil ameliorants
- **Researcher Outreach – Support for Molecular Assays**
    - Provide a pool of funds for researchers of current projects to access to begin using the molecular assays within their project for which existing budgets are prohibitive.
    - Use the fund to leverage engagement by researchers with the technology.
- **Producer Engagement**
    - Design and implement small projects in partnership with producers with specific topics of interest in pasture soil biology.
    - Utilise the interests of producers in soil biology to stimulate adoption of wide ranging best practice advances in grazing systems while using the molecular assays to monitor effects on key soil biological parameters.

A business case has been prepared to support extension of this work.

Contents

1 Background

1.1 Program Development

The genesis of Pasture Soil Biology Program emerged from MLA's Strategic Science Program which began in 2001 to invest in strategic basic science in support of developing enabling knowledge and technologies aligned with potential transformational change in the red meat industries.

Consideration of suitable areas for investment for Strategic Science identified soil biology as being one subject area for which the knowledge of the dynamic interactions between soil organisms, environment, plant species, pasture management and how to manipulate soils systems for soil biological health was much less in pasture based systems than in cropping systems.

The soil biology knowledge base for cropping systems had developed for over 40 years based on traditional and sometimes primitive investigative techniques which are very time consuming, labour intensive and often reliant on subjective interpretation, but was assisted by investigating monoculture plant based systems. Achievements by the pioneers in this research has been transformational in advancing the interests of the cropping industries where there is now reliable technology and knowledge available to effectively manage the risk of soil borne pathogens and better manage soils for better soil structure and nutrient cycling.

The challenge in pasture soil biology is to develop a similar body of knowledge but faster and within a much more complex biological system with mixed plant species and the interactions with livestock. Another challenge is to achieve this in a technical environment of declining Australian expertise in traditional soil biology and plant pathology.

1.2 Knowledge and Opportunity Audit – Soil Biology

A review of Australian pasture soil biology research was commissioned by MLA in 2002 to evaluate current knowledge and indicate potential areas for research investment which would translate to benefits to the grazing industries (Gupter and Ryder, 2003).

The soil biology audit and additional contributions from others suggested that there are significant knowledge gaps in:

- Understanding the nature and degree to which soil based pathogens impose biological constraints on pasture productivity across the range of agro-ecological pasture zones of significance to the grazing industries.
- How pasture management can be manipulated to favour improved soil biological functions for improved pasture productivity
- How temporal dynamics of carbon availability (organic matter, roots, root exudates) interact with key soil biological functions in response to different pasture management strategies
- Defining the number of days, and when, within seasons in which microbial activity is optimised in order to predict soil biological function

- Understanding the way in which key soil and plant-root rhizosphere microbiological processes can enhance pasture plant recovery from grazing and tolerance to root pathogens or other stresses.
- Understanding how soil food web dynamics can be influenced through pasture and grazing management to synchronise nutrient mineralisation with plant demand. The aims here are to enhance nutrient efficiency and reduce soil acidification through nutrient loss or accumulation of nitrogen.
- Understanding the role of grazing management on seasonal soil nutrient cycling within grazing systems to enhance biological release of soil and organic matter bound nutrients.
- Understanding of disease biology in pasture systems, particularly with non-legumes, disease control measures and development of pathogen DNA probes as diagnostics for important fungi and nematode pathogens
- Understanding critical soil biological processes and conditions which favour optimal transitions between crop and pasture phases.
- The role soil biota can have in reducing carbon, nutrient and agrochemical pollution of catchment water.
- The impact of agrochemicals on soil biological groups, both pathogens and beneficial
- Pasture fertiliser interactions with soil biological processes and improved pasture growth
- The potential for soil macrofauna to enhance soil condition for pasture renovation in interaction with grazing pressure.

Recommendations from the Knowledge and Opportunity Audit were:

1.2.1 Problem Diagnosis

- Diagnose pasture productivity constraints using **Water Use Efficiency** measurements to compare actual pasture productivity to **potential productivity**, and determine the nature of the constraints (both for research and on-farm). Aim to set pasture productivity benchmarks.

- Determine the likely positive contribution of changing management of the system (e.g. increased carbon and other inputs) in achieving potential pasture production, via improved soil biological functions such as nutrient cycling, nutrient use efficiency and disease suppression. This will require several growing seasons.

1.2.2 Driving soil biological activity

Carbon inputs & availability

- Soil carbon availability drives or constrains soil biological function in Australian soils. Determine the temporal dynamics of **carbon availability** (seasonal, field-based, comparing management regimes, within pasture & pasture/crop systems) and link this to key soil biological functions. Depending on agro-ecological zone (e.g. mallee cf western slopes) the key biological functions will differ (nutrient mineralization and loss, pathogen survival, pesticide degradation, soil aggregate formation). Management regimes include grazing systems, pasture composition, pasture renovation and soil ameliorants.

- Determine the role of grazing management in carbon dynamics, in relation to soils as carbon sinks and global greenhouse gas budgets. (Research in the USA, for example, is far ahead of Australia, and we cannot "import" these results).

Water availability & temperature

- For key soil microbial processes (e.g. mineralization of N or development of disease suppression), define the number of microbially-optimal days based on conditions (soil type, soil moisture & temperature, based on weather data) found in different agro-ecological zones. **Aim**: to provide information for prediction of soil biological function.

1.2.3 Grazing management

- Plant re-growth can be used as an indicator for resumption of grazing. Test the link between plant re-growth and key soil and rhizosphere biological processes. Does this method of scheduling grazing also deliver greater benefits from soil biological activity? (i.e. long-term sustainability of soil biological processes such as nutrient cycling).

- Appropriate grazing pressure could stimulate pasture re-growth via rhizosphere biological processes. Investigate rhizosphere processes over a range of grazing pressures that may lead to positive feedback on microbial mineralization of nutrients and their availability to plants (resulting in more rapid pasture re-growth). (Basic research).

- Different grazing systems and grazing pressures lead to differences in soil biological functions (e.g. proportion of beneficial to deleterious organisms, nutrient cycling, disease expression or disease suppression). Determine the links between the composition and activity of soil biota under different grazing systems (linking soil biodiversity to function). (Basic research).

- Determine the impacts of grazing management on soil food web dynamics, importance of various trophic groups in different agro-ecological zones and links to biological functions (dryland, lower rainfall). For example, food web dynamics in relation to synchronization of nutrient mineralization with plant demands. When plant demand is low, nutrients can be lost. If demand and supply of nitrogen do not match, excess accumulation of N could contribute to soil acidity problems.

- Minimize nutrient losses from grazing systems, especially in higher rainfall areas. (Inappropriate grazing pressure could result in nutrient loss from the system via denitrification and by leaching). Determine the role of grazing pressure in soil nutrient loss (especially N, but also P in high rainfall areas). Determine regulators of biological nutrient mineralization and loss, as affected by pasture composition: how can soil biota activity minimise the loss of nutrients from pasture soils? **Aim**: recommend grazing management to improve efficiency of resource use & reduce off-site impacts.

- Investigate the relationship between carbon inputs (seasonal, above- and below-ground) and biological processes associated with soil nutrient cycling within a field-based grazing system. **Aim**: to determine the balance between input of nutrients versus nutrients provided by soil biological activity, to gain more benefit e.g. mineralized nitrogen, also P availability in

calcareous soils.

1.2.4 Management of pasture – crop transitions

Management of the transition from crop to pasture

- Seedling establishment and growth are important in the establishment of pasture and are affected by, for example, pathogen and nutrient status of soils. Develop measures of the status of key soil biotic activities in the transition from crop to pasture, to determine the impact of cropping phase management (e.g. nutrient cycling and availability, disease suppression). This is to provide information for farmers to maximize benefits from improved pasture soil biology.

Management of the transition from pasture to crop:

- Develop methods to evaluate a pasture soil prior to the next crop. Pastures provide a biologically-based benefit for the next cropping phase. For example, knowledge on disease potential, disease suppression potential, nutrient supply potential and soil aggregate stability at the end of a pasture phase will assist in deciding management practices for the next crop.

1.2.5 Soil-Borne Pasture Diseases: their Diagnosis and Control

- Determine the major soil-borne plant pathogens for non-legume pasture plants (mainly grasses, including perennial and native grasses; region-specific; following from diagnosis of biological constraints).

- Develop and deploy disease control measures, including chemical and biological treatments, for major soil-borne pathogens. Field-testing and assessment of potential for commercial development of bio-agents that induce systemic resistance to disease in pasture legumes.

- Develop diagnostic DNA probes for the most important pathogens (fungi, nematodes; new research tools and methods for on-farm management of diseases). To be useful, this must be linked to information on the effects of environmental factors on disease expression.

- Investigate the potential for development of disease suppression by promoting native microbial communities in pasture soils (i.e. control of disease by soil biological and/or physical factors while pathogen is present).

1.2.6 Removing negative impacts: Pesticides and pollution

- Establish the capacity of soil macrofauna such as dung beetle species to reduce pollution of water by pathogens and organic material (carbon and nutrients) that move from pastures into water catchments. (Determine compatibility with agro-chemical use).

- Determine the effect of agrochemicals on specific biota e.g. effect of anthelmintics on soil macrofauna, aiming to minimise collateral mortality.

- Determine the effect of new generation herbicides on plant disease expression and nitrogen fixation.

1.2.7 New Management options: System inputs and Pasture renovation

- Determine the full beneficial effect of pasture fertilizer inputs on soil biological activity (extent and duration of change in biological function), both directly and via improved plant growth. Consider this research alongside determining the benefits of greater carbon inputs.

- Pasture renovation to overcome soil compaction problems: compare the effect, benefit and cost of two contrasting approaches, i.e. soil physical disturbance versus changed grazing management, in different regions, for their ability to improve pasture soils and pasture productivity, especially via macrofauna activity.

The report made special note that in addition to established methods in soil biology, the application of new tools to investigate soil biota and their activities will be valuable for progress. The use of these tools, based on advances in molecular biology and biochemistry, should aim to contribute to the research goals and priorities suggested in this report.

1.3 Evidence of Pasture Soil Biological Constraints

To add to the Knowledge and Opportunity Audit, a brief literature review revealed a number of experiments conducted in Australian pasture soils over the last 20 years which have demonstrated significant pasture growth responses to soil applications of selected fungicides and nematicides (Table 1).

Table 1
Examples of effects of biocide drenches on pasture productivity

Location	Pasture	Treatment	Change
Mallee, 98 Various sites	medic	F+N+P+Zn	↑ 62% DM
West Vic, 85 Clunes	Sub clover	F	↑ 95% DM
Sth Slopes, NSW Holbrook,90	Sub clover on acid soils	Lime P F	↑ 12% DM ↑ 12% DM ↑ 10% DM
Sth NSW, 85 Wagga	Sub clover	F	↑ 58% DM
Nth Vic, 83	Irrigated Sub clover	F	↑ 96% DM
Palmerston Nth NZ, 92	Red clover	F	↑ 50% FW
Waikato NZ, 2000	White clover	N	↑ 40% DM clover ↑ 135% N fix
Timboon, Ellenbank SW Vic, 96	White clover	N + F	↑ 220% DM in white clover
Nth east USA, 73	Red clover	F	↑ 69% DM ↑ 260% plants m^2
Code: F = fungicide, N = nematicide, P = phosphorus, Zn = zinc			

While this evidence appears compelling that experimental soil treatments to remove soil-borne pathogens can dramatically increase legume based pasture growth, there is the possibility that the results may be confounded by the release of mineral nutrients from decaying soil microbial organisms destroyed by the biocides. Hence while it seemed clear that pasture plant root pathogens induced significant production loss, the true extent of soil biological constraints to pasture productivity remained open to question.

Other studies which focussed on pasture plant diseases have identified a wide range of pathogenic organisms associated with poor performing pastures. However these studies have usually not defined the key pathogen complexes responsible for poor performance and related these pathogens directly to production loss. Failure to define the nature of the biological constraints has made it difficult to develop effective management strategies.

Also, previous studies have strongly focussed on pasture legumes and neglected the pathology of improved pasture grass species

In addition to the above, the “Pasture Theme” elements of the Sustainable Grazing Systems (SGS) National Experiment supported by MLA during 1996-2002 indicated that grazing systems pastures were producing to around 40% of potential water use efficiency.

Much of this lost productivity remained unexplained in the SGS report other than suggestions of ineffective water retention in pasture systems, out of growing season rainfall events and low temperatures contributing to low water use efficiency.

Evidence from the pasture research literature suggest that poor pasture establishment, incidence of diseases, inadequate nutrition and weed competition are also key factors associated with low water use efficiency of pastures.

Political and community concern for efficient utilisation of available water and other natural resources in agricultural production systems is intensifying. Pressure on the grazing industries to improve returns per unit of water used relative to other forms of land use is also likely to increase significantly.

1.4 Program Aspirational Target

The evidence summarised above suggests that there are substantial productivity gains possible if research can help understand critical soil biological processes which then lead to development of pasture management strategies that favour soil biological health under pastures.

MLA models indicate that a modest overall increase in pasture productivity of **10%** would contribute a minimum net benefit of **$260 million** per annum to the grazing livestock industries of Australia.

The conclusion was that this improvement in pasture productivity would be eminently achievable at a probable very high return on investment and it was on this basis that the Pasture Soil Biology strategy was developed.

1.5 Program Strategy Development

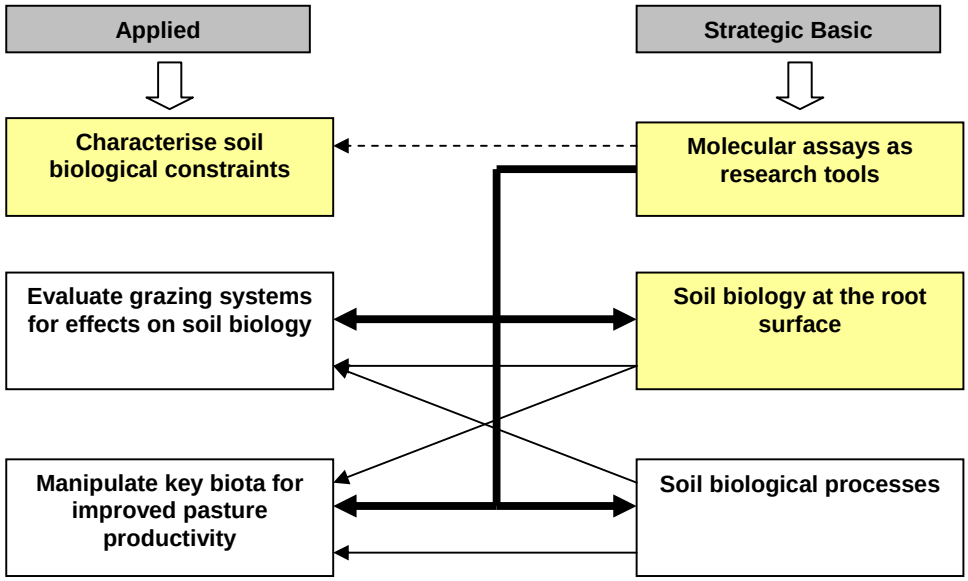
It was clear from the investigations above that the level of knowledge of soil biology under Australian pastures was significantly behind that from cropping systems which had benefited from over 40 years of dedicated research effort resulting in much transformational and beneficial knowledge and practices which have dramatically improved crop production across diverse cropping environments.

Based on the above information, a long term program strategy was developed with a view provide an R&D pathway to be achieved over a series of phases of R&D investment periods. A balance between strategic/basic and more applied research was envisaged. An overview of the strategy is described in Figure 1 and a more detailed description in Figure 2.

The essential features of the longer term strategy envisaged in a series of phases were:

- Develop research tools to enable accelerated research
- Use research tools to understand the dynamics of soil biology in grazing systems
- Based on this understanding, develop practices which favour soil biological health
- Undertake complementary strategic-basic research to fill critical enabling knowledge gaps.

Figure 1 Soil Biology Strategy from 2003

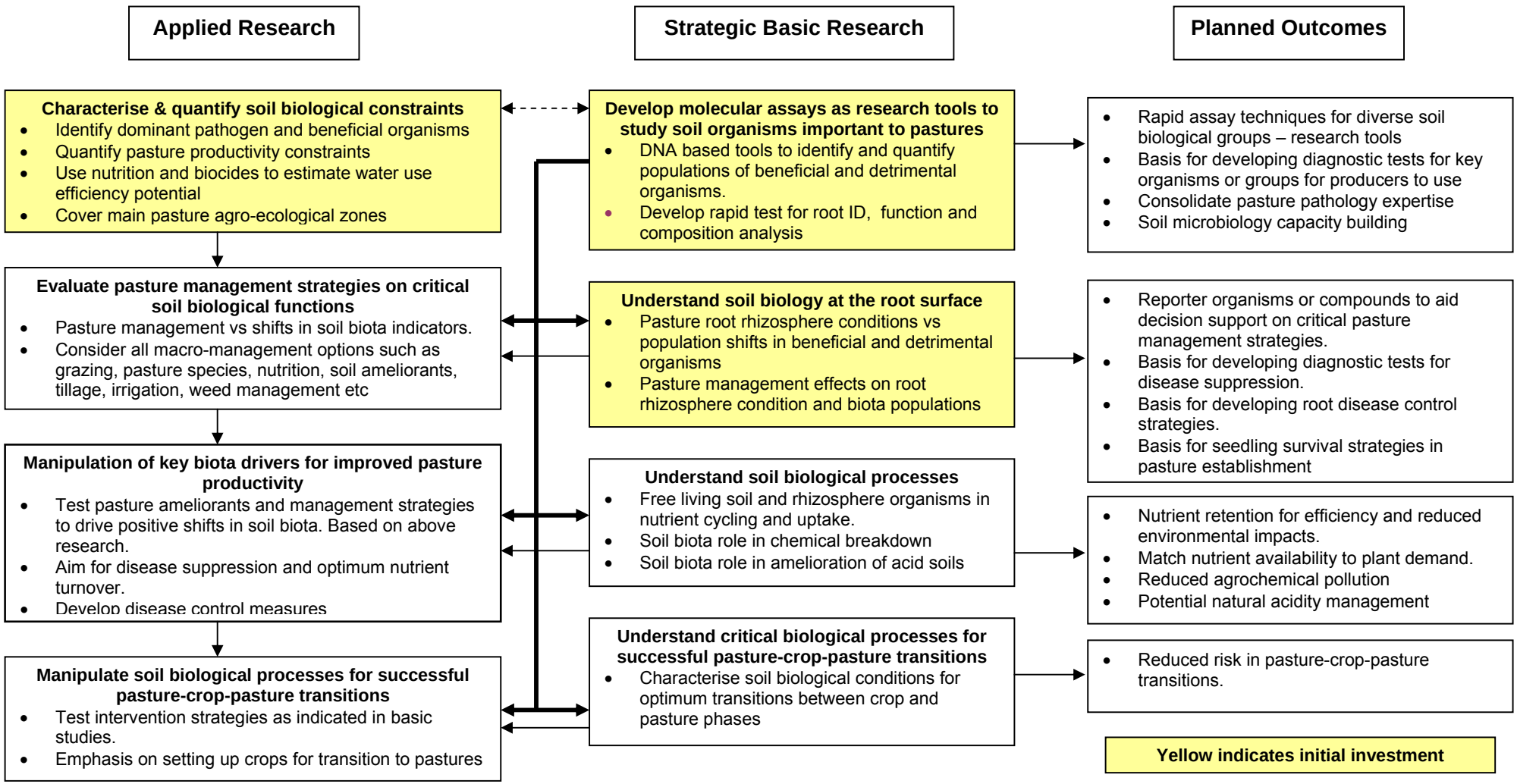


The highlighted boxes (▭) indicate the planned initial areas for investment and are placed in the context of planned future research framework required to achieve the aspirational target for the program.

Based on this strategy, co-investors were sought and project agreements developed.

Figure 2

OBJECTIVE: ***Soil Biological Health for at least 10% Improvement in Pasture Productivity***



2 Program Objectives

The overarching objective of the Pasture Soil Biology Program is to develop enabling knowledge and technologies, consistent with MLA's Strategic Science Charter, to:

- accelerate the research and development into diverse aspects of pasture soil biology
- acquire new knowledge on the interactions between soil borne organisms/functions, environment and management across the most important grazing systems relevant to the sheep and beef industries
- deploy the new knowledge to develop management practices favouring soil biological health and underpin achieving the aspirational target of at least an overall 10% increase in pasture productivity with the concomitant opportunity to enhance livestock productivity and health as well as the sustainability and environmental sensitivity of southern Australian grazing systems.

Phase 1 of the Pasture Soil Biology Program was initiated to:

- Develop molecular assays to identify and quantify selected soil borne organisms and functions for pasture soil contexts. The intention was to use these initially as research tools with a longer term view to make them available to producers and advisors and diagnostic and monitoring assays once sufficient knowledge is developed to interpret the tests (taking into account season x system x management interactions) and assign management tactics as has been done for the cropping industries.
- Validate the degree of soil borne biological constraints in some southern Australian pastures
- Develop a better understanding of the interactions between rhizosphere colonising micro-organisms and the changes in rhizosphere environment induced by plant stress.
- Later in this phase, begin interactions with researchers to introduce to, and educate in, the use of the molecular assays in pasture soils research. That is, commence a "delivery" process.

3 Program Governance

3.1 Research Alliance

There were three investors in the Pasture Soil Biology Program. MLA and Australian Wool Innovation Ltd (AWI) were equal major investors and GRDC a minor investor. The Dairy Research and Development Corporation (DRDC) was also approached but declined.

GRDC's involvement was sought to ensure that the Pasture Soil Biology Program was sensibly linked with the GRDC Soil Biology Initiative which was current at the time but with primary focus on soil biology in cropping systems.

An agency agreement was developed to underpin the governance of the Program and a management committee formed with representatives of each investor to over see the development and progress of the Program.

The committee met at least 6 monthly and included occasional site visits to Program Team meetings and laboratories.

The investment decisions and contract approvals were facilitated through the management committee.

MLA was the lead agency through which contracts and project administration was facilitated.

3.2 Program Investment

A total investment in the program was $2.65m over 4 years from July 2003:

- MLA $1.125m
- AWI $1.125m
- GRDC $0.4m

3.3 Program Management

Dr R J Hannam (R J Hannam & Co Pty Ltd) was appointed as Program Coordinator and as executive officer of the Program management committee.

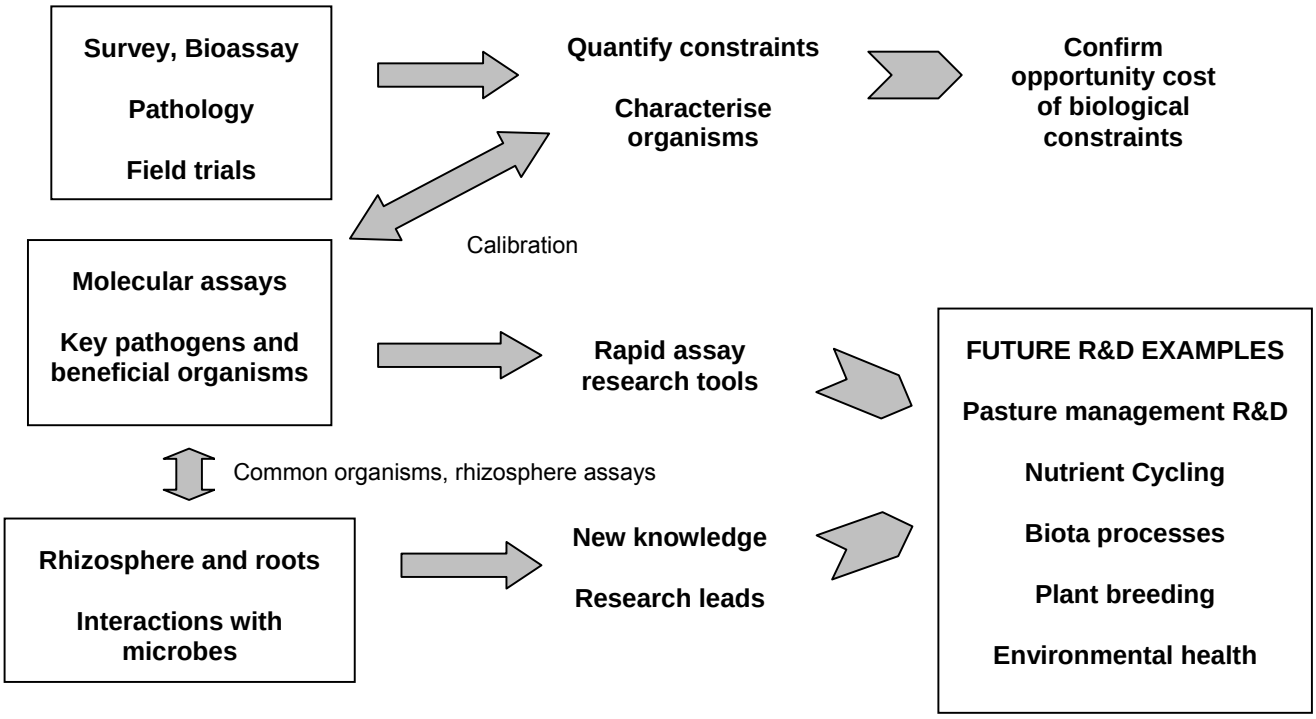
The tasks, on a part time basis, were to:

- Broker development of projects
- Facilitate synergistic linkages between projects and researchers.
- Act as the point of first contact on all matters pertaining to the projects
- Supervise delivery of research outcomes against contracted milestones
- Undertake site visits to assess research progress and proficiency
- Develop linkages with relevant soil biology research outside this program
- Represent the “alliance” at agreed forums and meetings on matters pertaining to pasture soil biology research
- Facilitate communication within the RDCs and externally to producers, advisors and researchers
- Oversee preparation of reports and press releases.
- Organise an annual forum for researchers to encourage linkages, communication, exchange of ideas and development of synergy of effort.

4 Program Projects

In alignment with the over-arching Program investment strategy described in 1.5, initial project commitments were developed within the following framework:

Summary of Initial Investment Strategy and Project Linkages



These are described in more detail below.

4.1 Characterise Soil Biological Constraints Affecting Pastures

An initial experimental effort sought to build on, and verify, Australian literature evidence of soil biological constraints to pasture productivity. The plan was to:

- Undertake a survey of soils in two geographically dissimilar regions in northern NSW and Lower south east South Australia : south western Victoria.
- Subject the collected soils to laboratory based bioassays for soil borne pathogens and a range of chemical and biological assays
- Use this information to select some field sites for more intensive studies where selected biocides were to be applied over a series of seasons to quantify the limitations imposed on pasture production by soil borne pathogens.

This effort resulted in a series of projects aimed at evaluating and defining the nature and extent of soil biological constraints to pasture productivity as well as providing a general examination of some elements of soil biology under pastures.

4.1.1 SHP.002 – Characterisation of Pasture Soil Biology Constraints – Northern NSW

Author – Dr GM Lodge (DPI NSW)

Report Summary

This project aimed to quantify soil biological constraints under pasture systems in northern NSW. It is one of two such projects, with the other project being based in south-east South Australia and western Victoria. The projects' specific objectives were to:

1. Identify pasture sites and characterise pasture performance on the Northern Tablelands and North-West Slopes of NSW and collect soil and plant samples for use in bioassays and analysis in quantifying and characterising soil biological constraints to pasture productivity and prioritizing key organisms as targets for development of molecular assays.

2. Provide field sites for validation and calibration of molecular assays of significant detrimental and beneficial organisms in pasture soils.

Twenty pasture sites were identified on producer's properties on the North-West Slopes and Northern Tablelands of NSW in conjunction with local District Agronomists. Target species at these sites were subterranean clover (*Trifolium subterraneum* L.), phalaris (*Phalaris aquatica* L.), and lucerne (*Medicago sativa* L.) with the perennial grass-base being either phalaris or native grasses. In each of the 20 paddocks, 3 locations were selected (~5 by 5 m) and herbage mass (kg DM/ha) was estimated and soil (0-15 cm) samples collected for further study. Soils were subsampled and sent to Dr Graham Stirling, Biological Crop Protection, Brisbane for nematode analyses, South Australia Research and Development Institute (SARDI), Adelaide for bioassay and identification of pathogens and commercial laboratories for soil chemical analyses and microbial biomass carbon (C) and labile C. For the 60 samples, a total of 35 variables were examined by comparing mean values ± standard errors and correlation analyses.

No major symptoms of fungal pathogens were observed at any of the sites, although DNA analyses of soil samples indicated that crown rot and common root rot were the most widely distributed fungal pathogens tested. Although all of the surveyed sites had a history of fertiliser application (generally single superphosphate, P or S) soil P Colwell levels were suboptimal at about 40% of the surveyed sites. Mean level of soil S was 6.9 mg/kg for all locations, marginally above the critical value (6.5-6.7 mg S/kg). However, all of the lucerne dominated sites had a mean S level of 5.2 mg/kg. Chemical analyses indicated that salinity, sodicity, and acidity were not limiting pasture production for the sites sampled.

Numbers of free living nematodes microbial activity and microbial biomass C were lowest for locations where lucerne was the main species compared with those sown to phalaris or subterranean clover. Mean potassium (K) levels were around 350 mg/kg of soil, but varied markedly from 95 to 840 mg/kg of soil. Phalaris sites had the highest mean levels of P and S (48.0 and 10.9 mg/kg, respectively). In contrast, lucerne sites had the lowest mean levels of DTPA Zn (6.4 mg/kg), DTPA Mn (29.7 mg/kg) and DTPA Fe (16.3 mg/kg), but the highest levels of exchangeable Ca and Mg (16.3 and 4.7 meq/100 g soil, respectively).

Mean total microbial activity was 1.3 µg FDA/g of soil.min and mean total C was 20.2 mg/g of soil. Low values for all of the measured biological properties were associated with lucerne pastures compared with the other pasture types. Since 30% of the surveyed pastures were dominated by lucerne, this was one of the main factors associated with differences among sites.

Total C and labile C were significantly and positively correlated r=0.96 (R^2 =0.9194, n=60). This strongly indicated that total C, which is more easily measured than labile C, may be a simpler indicator to use for initially screening large numbers of soils or detecting differences in the C status of soils from such pastures. Microbial biomass C was also significantly correlated with total and labile C (r=0.64 and r=0.66), but accounted for <45% of the variation in these biological properties. However, total microbial activity and microbial biomass C generally accounted for <42% of the variation in both total and labile C. Mean C: N ratios were 12.2: 1, with values always being <20:1, indicating that soil N mineralisation was likely to be occurring.

Across all locations and species there were no discernable correlations (r≥0.50) among nematode species, or between nematode species and any of the variables measured. However, within pasture species lesion and stunt nematode numbers were correlated (r = 0.57) for lucerne and stunt and cyst nematodes were correlated (r = 0.50) for phalaris pastures. There was a strong relationship (r =0.96) between soil K level and phalaris herbage mass, indicating that levels >500 mg/kg were required for high production. If soil K levels were <200 mg/kg, then phalaris herbage mass was <1000 kg DM/ha, indicating that the K nutrition of phalaris pastures in northern NSW may require further investigation.

Highly pathogenic genera of plant-parasitic nematodes (e.g. *Heterodera* and *Meloidogyne*) were not widespread, and population densities of moderately pathogenic genera (e.g. *Pratylenchus*) were relatively low. Responses to nematicide treatment were not obtained in the field.

Roots of pasture plants were generally relatively healthy. When damage was observed (e.g. root rot on sub-clover caused by *Pythium*), damaged roots were often replaced by a healthy new root system.

A series of field studies were undertaken in 2004 to gain further insights into potential soil biological constraints in the pastures of northern NSW by measuring field responses to fungicide and nematicide applications in a range of annual and perennial pasture species. A series of studies were conducted in winter and spring 2004 to:

1. Examine the effects of biocides on the herbage mass production of subterranean clover in the field,
2. Determine the efficacy of fungicides and dazomet on inoculum of 3 cereal fungal pathogens in the field,
3. Determine the efficacy of aldicarb on free-living and plant-parasitic soil nematodes, and,
4. Document the plant pathogens associated with subterranean clover in the field.

Drenching of biocides (both fungicides and nematacides) onto the soil surface was ineffective in reducing numbers of fungal pathogens and nematodes. No effect on pasture herbage mass (kg DM/ha) was recorded, although some evidence of phytotoxicity was observed. Etridiazole was particularly phytotoxic to both subterranean clover and native perennial grasses. Fungicides that

were phytotoxic did not always cause obvious above-ground symptoms. For example, above-ground symptoms were not observed on procymidone-treated plants.

Interactions that resulted in phytotoxicity occurred among fungicides. When used alone, azoxystrobin and mancozeb + metalaxyl were not phytotoxic, however, they severely affected plant growth when used together. If fungicides are to be used in future studies, it is recommended the effect of dosage on both the target plant and its pathogens must be determined. The capacity of the fungicide to move into the root zone with rainfall should also be confirmed for the soil types to which it is applied.

Dazomet incorporated to a depth of 15 cm killed fungal pathogens buried at a soil depth of 10 cm, but had no effect at 25 cm. This suggested that dazomet could be a useful biocide for work in pastures, as it killed soil-borne pathogens in the zone where it is incorporated. Under winter conditions in Tamworth, phytotoxic residues of dazomet dissipated in about 3 weeks.

Aldicarb had little impact on numbers of plant-parasitic nematodes in soil. In the field, a single nematicide application did not affect nematode numbers, while 2 applications reduced nematode populations only at some sites, but did not affect pasture herbage mass.

All the root systems collected from the field or from plants growing in potted field soil were relatively healthy. The most common symptoms observed were rotting and death of root tips, superficial cortical lesions on major roots, and flaccid, discoloured sections along roots. Occasionally, the initial taproot had rotted back to a stump, but a new and relatively healthy root system had grown from above the damaged area.

Fungi that were pathogenic to subterranean clover on agar were isolated from roots. The most common pathogens were *Pythium* and *Fusarium*, but an unidentified fungus also caused damage. These fungi need to be identified to species level and their pathogenicity on mature plants confirmed.

4.1.2 SHP.004 – Characterisation of Soil Biology in Australian Temperate Pastures with Particular Reference to Nematodes

Author – Dr GR Stirling (Biological Crop Protection)

Report Summary

This study was a component of a broader project which aimed to characterise the soil biology of Australian pastures and determine whether there were any major biological constraints to pasture production in Australia. The work focused primarily on nematodes because they are known to be useful biological indicators. Plant-parasitic nematodes are serious pests of many crops while free-living nematodes play a key role in important soil processes such as nutrient cycling.

Nematodes were identified and quantified in permanent and semi-permanent pastures at 108 locations in two contrasting environments; a summer rainfall zone in northern New South Wales and a winter rainfall zone in south-east South Australia and western Victoria. The pastures surveyed were based on four plant species, namely subterranean clover, lucerne, phalaris and perennial

ryegrass. Two plant-parasitic nematodes [lesion nematode (*Pratylenchus* spp.) and pin nematode (*Paratylenchus* spp.)] were found at about 50% of the locations sampled, while cyst nematode (*Heterodera* spp.) and root-knot nematode (*Meloidogyne* spp.) were present in localised situations. Lesion nematode (primarily *P. thornei* and *P. neglectus*) was considered the nematode pest most likely to be economically important on pastures, as it was widely distributed in the two study areas and is known to damage cereal crops in northern and southern Australia. Interestingly however, populations of lesion nematode in pastures rarely reached the levels usually observed following susceptible cereal crops, suggesting that pasture species were either less susceptible to the nematode, or that biological mechanisms of suppression are more effective in pasture soils than cropped soils.

In May 2004, experiments with the nematicide aldicarb were established at five field sites in northern NSW to establish whether plant-parasitic nematodes caused crop losses in phalaris, lucerne and subterranean clover pastures. Experiments were also set up in the glasshouse to measure the effect of rainfall on the downwards movement and efficacy of aldicarb. The results showed that aldicarb reduced populations of plant-parasitic nematodes in the field, but that efficacy was limited by inadequate rainfall. Nematode control was poor when nematode populations were measured in soil, but root counts showed that aldicarb was able to prevent endoparasitic nematodes such as *Pratylenchus* from multiplying in roots. Although herbage mass (measured in September 2004) did not increase following nematicide treatment, it is not possible to draw conclusions about the economic importance of nematodes in pastures from these experiments. Nematode populations in untreated plots were relatively low at two sites; phytotoxicity due to aldicarb was observed at the lucerne sites, and only 80 and 200 kg/ha of subterranean clover dry matter was produced at two sites where subterranean clover was expected to be the dominant pasture species. Further work is therefore needed to confirm the pest status of plant-parasitic nematodes in Australian pastures.

Free-living nematodes proved to be a useful indicator of soil biological status, as results from the survey showed differences in the nematode community between the two study areas and also between pasture species. Numbers of free-living nematodes were highest in the winter rainfall zone, while in the summer rainfall zone there were more free-living nematodes in subterranean clover and phalaris pastures than in lucerne pastures. Relatively low enrichment and structure indices in the summer rainfall zone showed that soil food webs at many sites in this zone were degraded. Food webs in the winter rainfall zone had higher enrichment indexes, indicating that more resources were available to support soil organisms. Climate had a major effect on the channel index, as the mean for the winter rainfall zone was 28 compared with a mean of 72 for the summer rainfall zone. This indicates that the detritus food web is bacterial dominant in the south and fungal dominant in the north.

When subterranean clover was grown in pots containing untreated field soil, gamma irradiated soil, soil heated to 65˚C, or soil treated with aldicarb or various fungicides, significant growth responses to aldicarb, heat and gamma-irradiation were observed. In contrast, many of the fungicides were phytotoxic. In another experiment with phalaris, heat treatment increased plant biomass by 66%. These growth responses to broad-spectrum soil treatments suggest that root pathogens capable of reducing the growth of pasture species are present in soils used for pasture production in northern NSW. Isolations of fungi from root lesions on subterranean clover at several sites yielded *Pythium*, *Fusarium* and an unidentified fungus, and all isolates produced disease symptoms on plant roots in preliminary pathogenicity tests.

The results of this study suggest that from a biological perspective, soils used for pasture production in the two study areas were reasonably "healthy". This conclusion is based on the relatively low numbers of plant-parasitic nematodes and relatively high numbers of free-living nematodes in most soils. However, this conclusion does not mean that the biological status of all surveyed pasture soils is satisfactory. For example, numbers of free-living nematodes in the summer rainfall zone were lower in lucerne pastures than in phalaris or subterranean clover pastures. At the level of an individual farm, measurements taken at three locations in each field indicated that there was often considerable variability in the nematode community within fields. The enrichment index, structure index and channel index often varied by more than 100% within a field, and at some sites, this level of variability was observed for two or three indices. More detailed studies are required to understand the causes of this within-field variation and quantify its economic and environmental impact

Comments

The outputs from these projects were combined in a scientific paper submitted to the Australian Journal of Soil Research - Stirling GR, Lodge GM (2005).

During the course of these experiments, the limitations in effectively using soil applied biocides to quantify biological constraints in the chosen field soils became clear. Uneven and ineffective distribution of the biocides within sufficient soil profile, plus phytotoxic effects on plants made it difficult to reliably evaluate in-situ biological constraints in these environments.

Also, data from the laboratory based bioassay experiments were rendered unreliable as a senior Australian pasture plant pathologist (Dr Martin Barbetti, University of Western Australia) revealed that soil disturbance during soil sample collection can dramatically alter the nature of the soil biology in the samples and impact on likely responses in empirical experiments.

Hence a new approach was conceived to develop an in-field bioassay technique to better evaluate biological constraints to early season germination and growth of sub-clover as a test plant. This commenced in 2005.

4.1.3 SHP.017 - Root Damage on Subterranean Clover in Autumn- Winter - 1

Author: Dr R Simpson (CSIRO, Plant Industry, Canberra) in collaboration with Dr M Barbetti (University of Western Australia) and Dr IT Riley (SARDI)

Report Summary

Numerous studies report pasture growth responses to fungicides, nematicides and/or fumigation treatments suggesting that some aspects of soil biology potentially constrain pasture production. Root rot fungi are likely to cause such a constraint. They have been known since the 1960's to occur widely in subterranean clover pastures and large claims are made about the extent to which they reduce pasture yield or even cause legume failure. However, most studies that indicate yield increases have been under artificial or experimental conditions and it was unclear whether the results can be extrapolated confidently to production in grazed paddocks. Early work in the Pasture Soil Biology Program also demonstrated that there are substantial problems when using biocide treatments to explore soil biology constraints. Biocides were only partially effective against the

target organisms, the efficacy of soil drenches was affected by soil type and/or the biocides were phytotoxic for plant growth. These results caste doubt on whether experiments reported in the literature reflected an unbiased sample of outcomes from studies of the impacts of soil biology.

An alternative “bioassay” approach was devised as a possible way to progress the assessment of potential for soil biology constraints in pastures. Seedling survival and damage to roots (similar to that expected of damping-off and root rot pathogens) was quantified after sowing a susceptible cultivar of subterranean clover (cv. Woogenellup) at 11 pasture sites/treatments in NSW, 4 in SA, 3 in WA. Lucerne (cv. SARDI 10) was the test species at 4 sites in the SA mallee. Annual ryegrass (cv. Safeguard) was also sown for comparison but grass root damage was not assessed.

New DNA probes for common root pathogens, some beneficial soil fungi and nematodes relevant to subterranean clover pastures were also employed to establish the prevalence of these soil microorganisms at the sites (see project SHP.005).

The first objective of this project was to test the application of the bioassay of early-season root damage. The bioassay approach was successful and indicated there is a widespread and substantial potential in autumn-winter for subterranean clover seedlings to either: (a) fail to emerge, or (b) to germinate and establish, but with a high incidence of damaged roots.

Failure of seedlings to emerge: There was significant early loss of both subterranean clover and annual ryegrass seedlings. Between 20 and 35% of subterranean clover seedlings were lost prior to emergence at a majority of sites. In severely affected pastures, 50-90% of clover seedlings failed to emerge.

Root damage: At every site, substantial root damage occurred on the subterranean clover and lucerne test plants that survived emergence and managed to establish. On average, only 34% of subterranean clover plants established with undamaged roots and in the best case only 60% of established plants had undamaged roots. This was despite soil moisture conditions across southern Australia in the 2006 autumn-winter period that were not expected to be particularly favourable for most root rot pathogens. Shoot yield of the plants was also adversely affected by root damage but it was outside of the scope and resources of this project to quantify the impact of seedling loss and root damage on pasture yield.

The project also aimed to provide a preliminary assessment of how DNA probes for plant species, common root pathogens, nematodes and some beneficial soil fungi may be used to indicate site characteristics and the presence and relative abundance of particular soil microfauna groups.

Presence of plants, pathogens and potentially-beneficial organisms was successfully quantified using the DNA probes. For the first time, it is possible to rapidly identify the pathogens likely to cause problems at different sites and to explore the ecology of organisms such as AMF which are difficult to study. Indeed, the extent to which AMF clades vary in their relative abundance between sites is essentially impossible to study using current technologies. The results presented in this report demonstrate that this technology has the potential to revolutionise the study of AMF ecology, infection of plant roots and soil biology in general.

Soil micro-organisms varied considerably between sites and the DNA probes enabled the sites to be characterised by their soil micro-organism profiles. For instance, some sites appeared to have only

a few pathogens whilst others possessed nearly all of the pathogens that can be detected currently. Some organisms, e.g. *Phytophthora clandestina*, were only present at particular sites; while others, e.g. *Pythium*, were universal. Developing a pathogen profile for a site by standard pathology methods alone would be difficult, time-consuming and potentially inaccurate because it is always possible to miss key species when trapping and culturing from infected roots. However, DNA probes only detect the organisms that they were developed for and will, therefore, be best employed in combination with standard pathology methods.

This study indicates that loss of seedlings (putatively to damping off and root rots) presents a large financial risk during pasture establishment even when good sowing practices are employed and emphasizes the importance of treating pasture seeds with fungicide prior to sowing. This technology is available now, but greater emphasis of the benefits of treating seeds should improve uptake of the technology. Development and deployment of diagnostic tests, such as the DNA probes being trialled in this project, will be a medium term task as some further development and testing is desirable. However, these tools have the potential to further reduce establishment risks because paddocks at risk can be detected readily and cheaply and it is also possible for the information to be used to guide plant cultivar selection or appropriate use of pesticide treatments.

It was obvious from the poor shoot vigour of test plants, that reduced pasture yield will be associated with root damage. Indeed, it is likely that sub-lethal infection and damage to pasture roots constitutes a large, but underestimated cost to production because it was so widespread (moderate to severe root damage occurred at every site) and because the damage occurs during autumnwinter when pasture yield limits stocking rate. Although grasses were not the primary focus of the present experiment, the data indicate that they may be similarly affected and further investigation is warranted.

Damping-off and root rot on subterranean clover has been recognised across southern Australia for 50 years and some progress has been made in selecting cultivars with increased field resistance to root rot. However, the extent of the root constraints observed in this study was alarming and it is clear that the true cost of autumn-winter root damage to production systems in unknown. It is necessary to investigate whether field resistance to root rots has developed in paddocks exposed to root pathogens over long periods of time, and to quantify the extent to which modern cultivars resist root damage organisms. It appears possible that pasture production and stocking rates could be lifted if it proves feasible to reduce or eliminate autumn-winter root damage.

The fact remains that insufficient is known about the impacts of soil-borne pathogens on pasture production in temperate Australian pastures. The levels of field resistance to soil-borne pathogens in our key pasture species are generally not known and the distribution and races of key pathogens in southern Australian pasture systems is poorly understood.

The present study used subterranean clover cv. Woogenellup, a root rot-susceptible cultivar, with the intention of assessing root damage (disease) “potential”. The bioassay approach avoided many problems encountered in earlier research and can now be adapted to:

(a) quantify the costs to pasture yield of root disease during autumn-winter;
(b) determine whether plants persisting in established pastures have developed resistance to root damage organisms;
(c) assess whether modern subterranean clover cultivars and key pasture grasses possess

reasonable resistance to root damage organisms; and to
(d) confirm the identity of the suspected causal organisms and/or organism complexes.

Estimating yield penalties due to root damage organisms will be difficult because few (if any) pesticides have broad spectrum action, often do not penetrate soils adequately, and fumigation treatments cannot be applied without killing the existing pasture and are imperfect because disease organisms reinvade fumigated soil. However, by combining the bioassay approach with use of the DNA probes to monitor pathogen reinvasion in fumigated soil, it should be possible to estimate the cost to yield of early-season root damage.

Comment

A poster summarising this work is in Appendices 10.1, 10.2.

A draft fact sheet has been produced to describe the field bioassay technique for evaluating soil biological constraints in pastures. This was developed based on suggestions from Dr Martin Barbettti, University of Western Australia.

4.1.4 SHP.0025 - Root damage and soil biology profiles of autumn-winter pastures

Author: Dr R Simpson (CSIRO, Plant Industry, Canberra) in collaboration with Dr M Barbetti (University of Western Australia) and Dr IT Riley (SARDI)

This project was a continuation of SHP.017 and, importantly, confirmed that root damage on pasture plants caused by soil borne root pathogens resulted in a c. 26% constraint on pasture production in the important autumn-winter period resulting in a significant opportunity cost in livestock production. It seems clear that the significance of this impact is as yet not fully appreciated.

Report Summary

Root damage on subterranean clover as a result of root-rot pathogens was identified many years ago and has been variously described as a substantial problem for clover yield or as an intermittent problem which can be severe in certain years. However, it is likely 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 true potential cost to production will go unrecognised.

A bioassay using subterranean clover cv. Woogenellup or annual ryegrass cv. Safeguard was tested and refined to further investigate the potential for root damage at four NSW sites and the impact of damaged roots on plant growth in the autumn-winter period. The bioassay methodology was shown to be robust and to provide a conservative but realistic estimate of the potential for root damage.

DNA assays for root pathogens and some beneficial organisms were also employed to understand their value in profiling soil biology at the sites and for identifying and quantifying pathogens in soil and damaged roots.

Generally speaking, failure of seedlings to establish and root damage on subterranean clover (cv. Woogenellup) was shown to be equally severe in two successive seasons. At one site only seedling

losses were considerably less in one year than another. However, substantial damage to roots was still observed. Across all sites, ~20-25% of seedlings did not establish and only 20-25% of test plants had “essentially-undamaged” roots. The largest category of clovers with damaged roots exhibited damage to primary lateral roots with about 10-15% of plants also having a damaged tap root.

The relative yield of shoots from plants in each root damage category was remarkably similar for all four sites. Plants with secondary lateral root damage achieved only about 80% of the growth of plants with undamaged roots, those with primary lateral root damage about 65%, and those with tap root damage only 55% of their potential yield. The results indicated a linear relationship between the extent to which roots were damaged and the growth that clover plants achieved during autumn-winter.

A new scoring system was developed to examine root damage on annual ryegrass and this revealed that problems with grass root systems were also prevalent, but a wider range in outcomes was observed between the sites than had been seen for subterranean clover. At one extreme, whilst 20% of seedlings failed to establish at least 60% of plants had a healthy root system; at the other, nearly 45% of the grass seedlings did not establish and less than 10% of plants had undamaged roots. Severity of root damage was also negatively correlated with plant yield. Annual ryegrass plants with root systems exhibiting brown tips or a few brown seminal roots achieved ~55-90% of the yield potential revealed by plants with undamaged roots, those with all seminal roots brown to black achieved ~35-80%, and those with nodal roots but seminal roots absent achieved ~30-65% of potential yield.

An estimate of the impact of root damage in pasture yield was made by assuming that pasture at each site was comprised of 34% clover: 64% grasses, and that clover and grass susceptibility and responses to root damage were similar to the bioassay test species. On this basis it was calculated that root damage may potentially constrain pasture yields 18% to 37% (mean 26%) during autumn-winter. Given the widespread occurrence of root damage and the fact that the autumn-winter period is a critical time of feed shortage, it is possible that this estimate indicates a significant problem for livestock enterprises across southern Australia.

The impact of root damage to grazing system productivity and enterprise profitability was estimated by simulation using Ausfarm and the GRAZPLAN pasture and animal models. For the purpose of the simulations it was assumed that root damage constraints only applied to autumn-winter production. The effect of root damage on net farm income was strongly influenced by stocking rate. A farm stocked conservatively (12 sheep/ha) and consequently achieving a relatively low income, was predicted to experience a significant but comparatively low financial loss (16%) when restrictions to autumn-winter pasture growth were moderate (i.e. <20% reduction in autumn-winter growth), but would suffer a 54% constraint on income if autumn-winter pasture growth were reduced by 40%. At higher stocking rates (15-18 sheep/ha) with potentially higher farm incomes, the impact of restricted autumn-winter pasture growth was more obvious with ~25% constraints on income for a 20% reduction in autumn-winter pasture growth and massive 58-84% constraints on income for a 40% reduction in autumn-winter pasture. Indeed, at pasture growth reductions above 20% the value of lifting stocking rate to improve net farm income was predicted to be progressively lost as a consequence of root damage and a grazier’s financial situation may even deteriorate seriously if root damage were constraining autumn-winter pasture growth by 30% or more.

The new DNA assays developed by SARDI Plant Diagnostics were invaluable in characterising the soil biology of paddocks. Although experience with them is still rather limited, they do seem to have provided a reliable profile of which organisms can be expected at a site. However, it was not clear at this stage how soil microbial DNA concentrations relate to the incidence of root damage.

Attempts to use the DNA assays to examine pathogen associations with root damage proved to be very enlightening and raised many new questions about whether root pathogens are acting alone or in concert, and the ecology of organisms and root damage in pasture soils. Using the DNA assays it was demonstrated that agronomic manipulations can disrupt clover root infection by particular soil-borne organisms raising the prospect that, through an understanding of the ecology of root pathogens in the rhizosphere, options may be found for managing root damage. At a number of sites, root pathogen concentrations on roots increased linearly with increasing root damage. However, at one site none of the DNA assays available currently were associated with root damage indicating a need to pursue development of DNA assays for other known root pathogens. The work highlighted the need to combine the DNA assays with standard plant pathology to confirm these new insights and to bolster interpretations of how soil fungi are interacting with roots and causing root damage.

The best way to explore whether management of root damage is feasible will come through work that combines use of the DNA assays, root damage bioassays and standard pathology so that an ecological understanding of how pathogens interact in pasture soils and cause root damage is obtained. Presently, there are no new recommendations that can be made about actions that graziers can take to manage or prevent root damage, other than to reinforce the value of fungicidal seed treatments at sowing and to suggest that some of the modern subterranean clover cultivars should offer some protection against constraints to autumn-winter productivity. However, it is important that further bioassays using “naturalised” strains of subterranean clover, modern cultivars and alternative clover species be conducted to ensure such advice is correct. It is also important to quantify the susceptibility of a broader range of the grasses on which graziers rely. This knowledge could go some way to formulating useful, early recommendations for graziers.

Comments

The DNA assays referred to in this report are reported more fully below in Section 4.2

The importance of this work cannot be understated as it reveals that both pasture legumes and grasses are significantly affected by soil borne pathogens, particularly in the grazing system critical autumn-winter period when the access to early feed is important for gazing enterprises.

The value of the DNA assays for soil borne organisms (in this case, pathogens) as research tools complementary to the field work is a strong outcome from the program.

So far this work has confirmed that significant soil biological constraints to pasture productivity due to soil-borne pathogens are common despite less conducive dry seasons and represent a severe opportunity cost to grazing systems enterprises, particularly during the critical autumn-winter periods. The proposition that the work continue to determine and validate suitable management practices for farmers to adopt to reduce these constraints is strongly endorsed.

A comprehensive scientific paper on this work has been submitted for publication.

4.1.5 SHP.016 – Role and Impact of Diseases Caused by Soil Borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems – A Review

Authors: Dr MJ Barbetti, Dr K Sivasithamparam (University of WA), Dr IT Riley (SARDI)

To add further to this assessment of potential biological constraints affecting southern Australian pastures, and objective literature review was commissioned and is summarised below.

Report Summary

Diseases caused by numerous fungal, bacterial, viral, and nematode pathogens singly and in combination (disease complexes) decrease pasture production and thereby adversely affect agricultural industries, in particular the meat and wool industries across southern Australia. Annual *Trifolium* and *Medicago* species in particular play an important agronomic role in dryland farming regions where they are often an integral component of cropping systems. They are particularly important in regions such as the south west of Western Australia, much of South Australia and parts of Victoria which have a typical Mediterranean-type climate where they grow as winter annuals that provide both nitrogen and disease breaks for rotational crops. Lucerne (*Medicago sativa*) is increasingly becoming an integral component of cropping systems in south west, southern, and south eastern Australia. While grass species (*e.g.*, *Lolium* and *Phalaris*) have been largely relegated to relatively minor importance by comparison, there is increasing interest in the potential benefits from grass species, including native grasses.

Necrotrophic soil-borne fungal pathogens dominate the south west, southern, and south eastern regions of Australia, and particularly the Mediterranean-type areas therein because of the ease of survival of these trash-borne pathogens on infested residues over the dry summer period and because of the impoverished and nutrient-deficient soils across many parts of these areas. As such that there is often relatively low microbial competition with either the necrotrophic fungal pathogens or soil-borne nematodes, predisposing the plant host to these diseases. Although there a many nematode and necrotrophic fungal pathogens recorded on annual and perennial forage legume and grass species, this report outlines only those associated with diseases caused by major and/or widespread soil-borne pathogens.

A number of nematode and necrotrophic soil-borne pathogens have been associated with significant productivity decline and pose a serious threat to one or more annual or perennial forage legume or grass species to the extent that they require reseeding. For example, for fungal diseases subterranean clover and/or on annual medics, *Phytophthora clandestina*, various *Pythium* species such as *P. irregulare* in particular but also *P. ultimum* and *P. spinosum*, *Aphanomyces eutichies*, *Rhizoctonia solani*, and one or more of various *Fusarium* species such as *F. avenaceum* in particular but also *F. acuminatum*, are of concern. Other important soil-borne necrotrophic pathogens on annual pasture legumes may also include pathogens such as *Phoma medicaginis* and *Cylindrocarpon didymium* in specific locations. If we are to manage the significant threat posed to legume pastures (particularly subterranean clover) by *P. clandestina*, there is an urgent need to reassess across southern Australia the race situation for this pathogen which has the ability to rapidly generate new races to overcome existing cultivar resistances. The search for genotypes with resistances to multiple pathogens needs to be intensified if there is to be improved management of

diseases where several different pathogens occur together in the field, which is almost exclusively the case across all pasture species utilised in southern Australia.

The association of *Fusarium* spp. with subterranean clover and annual medic roots, crowns and pods, and grasses, is cause for additional concern as a number of them have been shown to be responsible for the production of deleterious mycotoxins. Additionally, pathogens such as *Phoma medicaginis* are also known to stimulate production of phyto-oestrogenic compounds in some legumes to high levels that can adversely affect ovulation rates in sheep.

Approaches to disease control include a range of management strategies that have been utilised to varying degrees for control of necrotrophic soil-borne pathogens of pasture legumes. In particular, host resistance offers the most cost-effective, long-term control, particularly as useful resistance to a number of these diseases has been identified. Cultural control strategies, including grazing, manipulation of nutrition, rotations, and seed health, and the application of fungicides offer further opportunities for restricting losses from necrotrophic soil-borne fungal pathogens of annual and perennial forage legume and grass species, especially if applied as an integrated management strategy.

The role played by necrotrophic fungal soil-borne diseases may in fact be far wider and have greater impact than often first considered, as there are many instances where diseases on annual and perennial forage legume and grass species are also common to other rotational crops. Except for annual *Trifolium* spp., the physiological impact of most soil-borne diseases on annual and perennial forage legume and grass species has not been adequately quantified for south west, southern, and south eastern Australia. However, the full array of direct and indirect losses needs to be considered for the most important soil-borne pathogens, and not just herbage and seed yields. This, considered along with the potential for mycotoxin and/or phyto-oestrogen production, highlights the extent to which necrotrophic soil-borne pathogens can affect productivity of annual and perennial forage legume and grass species and far exceeds simple yield limiting components. The success and outcome with sourcing resistance in annual pasture legumes, such as annual *Trifolium* spp. and perennial *Medicago* spp., highlights the value of seeking out host resistance from the Mediterranean centre of origin, even if the particular diseases of interest frequently do not occur there, in the same way that has been shown for the evolution of herbicide resistances. This is an area of research that will allow development of new host materials containing multiple resistances/tolerances.

Much of the information on soil-borne pathogen-induced losses comes from experiments involving glasshouse, controlled environment, spaced plant or single row field plot studies. While showing the potential of a pathogen to cause damage, these are done under conditions that could be considered to be unrelated to what happens in grazed annual or perennial pastures and provides information that cannot be reliably extrapolated to them. There is a need to assess soil-borne fungal and nematode pathogen-induced losses in ways and situations that allow the data obtained to be used to make rational disease management and economic assessments. Grazed monoculture first year swards can provide such information, larger sized sown swards providing more relevant data than the commonly used simulated or mini swards. Relatively little work has been done to date in regenerated annual or established perennial swards and commercial pastures. This is probably because their use involves more complex assessments mainly due to the presence of more than one plant species.

There are a number of issues that, if addressed, should lead to improvement in the assessment of soil-borne fungal and nematode pathogen-induced losses occurring across southern Australia. It is desirable that the level and impact of individual soil-borne fungal and nematode pathogens and their complexes be defined throughout as much as possible of the geographic range of the pasture species they infect, within and between years, and include periods when feed is limiting. Herbage and seed yield losses should be assessed in grazed monoculture swards or, wherever possible, in mixed species swards or grazed commercial pastures, along with pathogen-induced changes in botanical composition, numbers of plants regenerating (especially for annual pastures), persistence after the first year, rotational effects (such as nitrogen availability) and factors affecting feed quality (such as phyto-oestrogens and mycotoxins). There would be significant benefit from improvement of economic evaluation packages or models, from making them more user-friendly and, in particular, from employing them more widely. There is a clear need for improved extension to achieve a wider appreciation of the need for the management of soil-borne pathogens and the benefits derived from it. Given the labour-intensive and long term nature of field work involved with diseases in pastures, the tendency of funding to be targeted at increasingly short term minimal expenditure projects needs to be addressed if the necessary studies, targeting issues such as soil-borne pathogen-induced yield loss, are to occur.

It is clear that nutrients affect the severity of disease not only by influencing root physiology and host resistance but also by affecting the interaction between host and pathogen and/or the antagonist, each of which can also be affected independently by the availability of nutrients. It is noteworthy that nutrients and food base requirements are more critical for biological control agents operating through ‘pathogen suppression’ than for those which are active only in ‘disease suppression’. It is important to understand the soil saprophytic behaviour of not only the individual soil-borne pathogens but also their antagonists in relation to nutrition and their physical environments before embarking on field trials in southern Australia. It is equally important that we are aware of the possibility not only of differences between the soil-borne pathogens and their antagonists but also the effects of various soil nutrient sources, especially of carbon and nitrogen. It must also be emphasized that saprophytic behaviour of bacterial antagonists are likely to be different from fungal antagonists especially in their responses to the abiotic environment of soil. Clearly, the critical role of nutrients in the nature of the inoculum carrier needs to be recognized as it is likely that in many cases, especially with 'pathogen suppression', failure or success of a biological control agent in the field may well depend on nutrient requirement. Improved management of diseases with nutrient amendments thus implies not only the enhancement of host growth and defences but also the provision of nutrient bases for the effective activity of the biological control agent in soils of southern Australia. This could in many cases be soil microflora which provide a microbial buffering against the pathogen(s), acting as a general antagonism to the pathogen(s). It is evident that little is currently known about the interaction of nutrition with the major soil-borne pathogens of most if not all pasture species important to southern Australia. Overall, there is significant potential for improved management of major soil-borne pathogens of pasture species across southern Australia from a better understanding the full potential for manipulating nutrition for improved disease control.

This report covers research and extension articles published on diseases caused by soil-borne fungal and nematode pathogens of annual and perennial forage legume and grass pastures which are sown in south west, southern, and south eastern Australia and highlights those currently believed to adversely affect the growth and/or production of the affected plant species. It also includes some of the animal toxicoses from toxin producing soil-borne fungi which in their own right

may or may not cause disease of the host species. This report in general does not cover soil-borne fungi and nematodes that are simply listed in records of fungal and nematode pathogens occurring on one or more hosts among the pasture species.

Comments

This report reinforced the expectation that soil borne pathogens can severely limit pasture productivity across southern Australia and also highlighted some knowledge gaps, with much less known about the impacts on pasture grasses and perennial mixed swards than mono-culture legumes; interactions between soil pathogens and pasture species composition; factors affecting feed quality due to toxic mycochemicals; the interaction between mineral nutrition and the prevalence and severity of soil borne diseases in pastures.

Dr Barbetti has also highlighted that losses from pasture root diseases can be categorised as:

Direct Losses	Indirect Losses
• Reduced plant growth • Reduced seed set • Reduced plant nutritional value • Reduced palatability • Reduced seed viability • Increased toxin production	• Poorer pasture composition • Reduced residual fixed nitrogen • Reduced utilisation of pasture inputs • Reduced effectiveness of crop disease breaks • Greater exposure to soil erosion risks • Greater costs of management

Two scientific review publications were produced from this work.

4.2 New Molecular Research Tools for Soil Borne Organisms

This component of the Program was a major effort to develop DNA based assays to identify and quantify targeted soil borne organisms in pasture soils.

It follows from their successful use in cropping systems where they are used to estimate the risk of soil borne diseases to mono-culture crops, primarily cereals for which a strong understanding of the disease organisms x environment x crop management interactions have been well established over 40+ years of research and excellent disease management practices well developed.

The intent is to develop similar molecular assays for important soil born organisms in pasture soils to accelerate the development of research and a body of knowledge for the more complex pasture systems as exists for cropping systems.

Once this knowledge is developed and best practice management practices developed, these assays will be valuable for producers and advisors to use to diagnose risks to soil borne pathogens and monitor the effects of management practices on soil biological health.

A key part of this effort is to engage with other field based researchers to introduce the new technology, educate them in some basic soil biology and encourage them to adopt the new molecular assays as research tools for their own work to better understand soil biology in their research experiments.

4.2.1 SHP.005 – Molecular Tools to Study Soil Biological Constraints to Pasture Productivity - 1

Authors: Dr IT Riley and Dr AC McKay - SARDI

Report Summary

This project has developed a series quantitative DNA assays to demonstrate potential benefits of using one technology platform to measure levels of different soilborne organisms concurrently in a soil sample; organisms that could be interacting to affect pasture production.

The initial targets included a cross section of plant pathogens including fungi, oomycetes and nematodes and selected beneficial organisms. Assays were also developed for key plant species to measure amount of roots in the same soil sample. The beneficial organisms targeted included arbuscular mycorrhizal fungi (AMF) which are very difficult to study using current techniques, and *Trichoderma* spp, which are often associated with disease suppression.

The project is based on the high throughput DNA extraction system developed by SARDI/CSIRO to process 500 g soil samples. DNA extracted from the soil represents all living cells in the sample including soil microbes and plants. Quantitative assays can be used to determine the densities of the target organisms in the original soil sample. This system was originally developed to monitor levels of soilborne pathogens of broadacre crops.

Previous techniques have constrained research on biological constraints in pastures. These typically involved washing plant roots from soil samples, separating them into different species and

scoring for disease. Many of the pasture diseases are caused by pathogen complexes and a variety of techniques were required to extract each organism. The nature of these techniques often meant they could not be used on the same sample. The DNA assays overcome these constraints, although a test is required for each target species.

Plant assays

The plant DNA assays are expected to revolutionise studies of plant root systems, especially in mixed swards. They have potential to provide a better measure of root responses to different management strategies and environmental stresses.

Assays have been developed for *Trifolium subterraneum*, *Phalaris aquatica*, and *Lolium/Festuca* spp. These assays are very sensitive, and in spiking experiments can easily detect one mg dry root and one seed per 500 g soil. Results from pot trials using ryegrass as the target species show changes in soil plant DNA levels are more responsive than changes in root dry weight. In pot experiments, soil DNA levels declined more quickly than root dry weight when the plants were subjected to treatments such as defoliation and herbicide application. These results support previous findings that DNA degrades quickly in dying cells.

The plant assays were also used in combination with other assays to detect treatment differences in field trials (SHP018). Work has also commenced on using the assays to monitor root distribution in the soil profile. Samples from 12 soil pits across southern Australia have been used to check the DNA extraction system can cope with different subsoils. So far significant extraction problems have only been encountered with subsoils at 2 sites.

Oomycetes and fungal plant pathogens

Work on plant pathogens has focussed on the oomycetes, *Phytophthora clandestina* and *Pythium* Clade F (which includes *P. irregulare*). Assays for *Rhizoctonia solani* AG2.1 and AG2.2 developed by a HAL funded project were also evaluated for use in pastures. These assays, along with those previously developed for broadacre crops, were used to assess pathogen levels in bioassay trials and pasture management trials across southern Australia by SHP.017 and SHP.018.

The assays were also used to determine which pathogens where present in diseased rots of sub clover and lucerne seedlings. The results show the DNA assays can detect pathogens that are difficult to isolate using standard plating techniques.

Plant parasitic nematodes

Assays were developed for *Pratylenchus penetrans*, a cluster of root knot species *Meloidogyne javanica/arenaria/incognita* and *Heterodera trifolii* (white clover only). These complement those developed by previous projects. Also new assays for *Meloidogyne fallax* and *M. hapla* are expected to be funded by HAL. These nematodes also attack sub clover, lucerne and white clover, but their incidence is not known.

The nematode assays were included in the suite used to assess the pasture trials in the higher rainfall areas, but detected surprising few nematodes (SHP.017 and SHP.018). More work is required to determine if there is are other species operating in these areas.

Beneficial organisms

Arbuscular Mycorrhizal Fungi (AMF). Research on this important group of fungi is constrained by lack of suitable techniques. AMF consist of a diverse range of genera, which cannot be cultured or distinguished in roots and are difficult to assess in soil. The potential of the DNA assays to overcome these constraints is creating a lot of interest amongst scientists.

A single test to detect all AMFs could not be developed; instead we had to develop a series of 5 tests. The specificity of each test has been confirmed by sequencing the PCR products from selected field soils. We still have to develop standards and expect this to be achieved within the next year or so, through collaboration with a SAGIT funded project supervised by Prof Sally Smith (University of Adelaide).

Despite the lack of standards, the assays were used to demonstrate treatment responses in the pasture trials assessed by SHP.018.

Trichoderma spp. Trichoderma is an important genus that is often associated with disease suppression in soil. Two assays are under development to cover the known species. These assays are still under development and have not yet been used to assess soil samples. We expect these will be ready for use within the next 12 months.

Beneficial nematodes. There is a broad range of nematodes in soil that perform different roles. They broadly fall into four groups, these that feed on bacteria, fungi, plants and other nematodes. The structure of the nematode population in soil can be used as an indicator of environmental impact. Considerable work was done in collaboration with SHP.002 to develop tests for each trophic group, but unfortunately the DNA sequence information did not allow a manageable number of tests to be developed. This work was deferred in favour of developing the tests discussed above.

4.2.2 SHP.018 – Pasture Soil Biology Molecular Assays – Pilot Delivery

Authors: Dr IT Riley and Dr AC McKay - SARDI

Report Summary

This project was developed to support delivery of new DNA-based assays to pasture research groups for use as research tools to study soil biological constraints to production and sustainability.

SHP.005 project has been developing molecular assays for a range of soil organisms including pathogens and beneficial organisms and key pasture plant species. The latter are being developed to provide a DNA option to assess root growth in soil. Concurrently, Dr Steve Wakelin, CSIRO Division of Sustainable Ecosystems, has developed DNA assays for soil biological function and community structure. A number of assays from both groups have reached a stage of development where they can be evaluated in field trials, and help determine if they need further refinement.

The combination of these assays is expected to provide powerful new tools to study biological constraints to pasture production.

To engage pasture researchers and gain valuable field experience, four pasture trials were selected to evaluate the most advanced assays. These included recently established EverGraze sites at

Wagga Wagga, NSW and Albany, WA, and two long established experiments including the MASTER site run by NSW DPI, Wagga Wagga and a grazing by phosphorus experiment run by CSIRO Plant Industry, Hall, ACT.

The results revealed significant treatment affects at each site. At the MASTER site a significant impact of lime was detected on levels of arbuscular mycorrhizal fungi (AMF) and several important plant pathogens including Take-all. While levels of some pathogens such as Pythium and *Rhizoctonia solani* AG2.2 were not affected by liming. This was correlated with sub clover soil DNA levels, which were also not affected by lime. Sub clover is a host of both pathogens. In the P X Grazing trial, the DNA assays detected differences in sub clover, *Pythium* and *Phytophthora clandestina* levels between treatments. Treatments differences reflecting the different pasture compositions were also evident in the EverGraze sites.

Use of molecular assays to assess plant DNA levels is soil, is a novel approach to monitor root growth. Combining assays for different plants, pathogens and beneficial organisms should provide a better insight to biological constraints impacting on productivity and water use efficiency, and ultimately facilitate development of more efficient and robust pasture systems.

The CSIRO assays for key functional genes and community analysis also detected significant changes in abundance of functional genes and species composition between the different treatments, which included liming, grazing rates, P application and pasture composition.

A pilot training course and draft manual was developed to support introduction of the DNA assays. Workshops were conducted at Wagga Wagga NSW and Albany WA. These were attended by 30 scientists from State agricultural research agencies and universities. Their backgrounds included senior pasture researchers, visiting scientists, research students and some senior technical staff. The material was well received and stimulated considerable discussion on how this technology could be incorporated in the research programs. The feedback from participants was constructive and will contribute to refinement of future training activities, and priorities for development of future assays.

Engagement with pasture scientists through this project has further highlighted the value of the DNA approach to provide a practical method to study soil biological constraints as part of development of robust pasture management systems. There is strong interest in seeing the technology grow and applied and particular enthusiasm for using the plant assays to monitor root growth in soil.

Further development and promotion of DNA assays as research tools is warranted

4.2.3 Sub Consultancy Report to SHP.018 – Molecular Microbiology in Soil Pasture Systems: Management based drivers of ecosystem change and function

Authors: Dr S A Wakelin – CSIRO Land and Water

DNA extracted from the soil samples collected at the various field sites was provided to Dr Steve Wakelin, CSIRO Land and Water to evaluate molecular assays capable of testing for specific soil biological functions and also microbial community analysis. These were used to round out the story in SHP.018 and also assess the future of pursuing the use of soil biological function assessments within the soil biology research effort.

Report Summary

Pasture based agri-indrustries have much to gain through the understanding and management of soil biological processes. Gains may be made either directly through increased production and / or indirectly through sustainability gains and reduced environmental impacts.

In order to manage soil microbial communities, methods to accurately detect and quantify changes in species composition and biological functions present in soil must be used. Recently, the revolution in molecular microbial techniques has allowed researchers to accurately measure changes in the species of bacteria and fungi present in soils (and other soil microorganisms) and also quantify the amount of microorganisms involved in key ecosystems process. Examples of these are nitrogen fixation (N-input into soils), antibiotic production in soil (biocontrol), C and S cycling and greenhouse gas emissions from N_2O and CH_4.

The first step towards being able to manage microbial communities for increased production, sustainability, or reduced environmental impact is to identify key agri-management processes affecting the range of bacteria and fungi in soil, and also determine which management practices affect the abundance of species involved in key functions.

This report describes the application of modern molecular microbial techniques to investigate key agri-management drivers of species assemblages and functions in pasture soils. The study examined changes in soil microbial communities at 4 long-term pasture-based field trial sites in Australia and provides, for the first time, information of how management practices at the sites are affecting soil microbial communities.

The results show that most on-farm management practices directly affect both the species of bacteria and fungi present in soil and also the quantity of soil microorganisms involved in specific functions related to N cycling. The most significant drivers were found to be liming of soil, which not only had a large effect on the types of microbial species present in soil but was also a key driver of functions within those microorganisms. Pasture crop type, P addition and, to a lesser degree, stocking rate also affected microbial species and their functions in soils. Changes in soil microbiology due to P fertilisation or stocking rate may have been due to changes in the pasture plant species at the trial sites.

Interestingly, a natural cycle of bacterial and fungal species in soil (change in communities over seasons) was detected at the Wallaroo site. This was despite the testing being conducted during a drought.

This report clearly demonstrates that farm management practices have a direct affect on soil microbial communities and the functions therein. As such, this identifies a potential of targeting key management practices, such as pesticide or fertiliser addition, to the most biologically-sensitive window (e.g. best use of N fertiliser / reduced N_2O-based greenhouse gas emission) for gains in production or environmental endpoints. The understanding of the changes will need to be put into a whole-of-system perspective if gains in production, sustainability and environmental issues are to be made.

A publication (Wakelin and McKay, 2007) based on this work has been submitted to the Australian Journal of Soil Research.

Comment

These projects were highly successful in demonstrating the potential power of the molecular assays for soil borne organisms, plant roots and soil biological function to discriminate between established treatment effects in a range of experiments.

In addition the assays for soil microbial function and community analysis indicated potential for use in research on the environmental impacts and greenhouse gas emissions from different grazing systems in response to management, plant species, climate and landscape differences. It is anticipated that tis would be a focus for any phase 2 of pasture soil biology research.

The training for researchers was well received although it is clear that it must be on-going and with sufficient follow-up information updates to convince researchers who have given little thought to soil biology or considered it an area of investigation that is too complex to embrace the power of these new research tools.

4.2.4 B.SHP.024 – Molecular Tools to Study Soil Biological Constraints to Pasture Productivity - 2

Authors: Dr IT Riley and Dr AC McKay - SARDI

Report Summary

This project continued key aspects of the work undertaken by SHP.005, SHP.017 and SHP.018 to develop and demonstrate the value of DNA assays to study biological constraints in pastures.

SHP.005 developed quantitative DNA assays for a cross section of soilborne pathogens of pasture plants, key plant species (to assess root growth in soil) and the beneficial organisms arbuscular mycorrhizal fungi (AMF) and *Trichoderma* spp. The latter is often associated with disease suppression.

The emphasis was on continuing field evaluation of the assays developed in SHP005, develop further the AMF and *Trichoderma* tests, and develop new tests to support collaborating Centre of Natural Resource Management (CNRM) and State funded projects studying biological constraints of lucerne in the Murray Mallee. To promote adoption of the technology the manual developed by SHP.018 will be updated and two more training workshops conducted.

Key objectives of the project were to:

- extend the range of DNA tests available for use in field trials to include further development of the AMF and *Trichoderma* tests, development of a new test for lucerne and also commence on developing tests for three important grass weeds, five pathogenic oomycetes and fungi, and one more plant parasitic nematode.
- apply existing and new DNA tests to field experiments conducted by this project and collaborators to study soil biology and demonstrate the value of the technology to assist development of management strategies to alleviate biological constraints to pasture productivity and sustainability.

- update and extend the training manual developed by SHP.005, to accommodate a wider range of tests and deliver training workshops to researchers in eastern and western Australia.

Test development
New tests for *Medicago sativa* (lucerne), *Hordeum* spp. (barley grass), *Vulpia* spp. (sliver grass) were added to the tests for *Lolium* spp. (ryegrass), *Phalaris aquatica* and *Trifolium subterraneum* (subterranean clover) developed earlier under SHP.005. These tests were found to be sensitive and of useful specificity and are ready for application to field studies. Lucerne was selected because it is an increasingly important summer growing perennial with the potential to improve water use by pasture and increase feed arability. The test for weedy species, *Hordeum* and *Vulpia*, will allow studies on their impact in interplant competition and in contributing to pathogen build up.

Test for fungal beneficials, arbuscular mycorrhizal fungi (AMF) and *Trichoderma*, were improved and refined. Three new AMF tests were developed to replace two tests developed under SHP.005 and an addition *Trichoderma* test was added to give a better coverage this disease-suppressing group.

The technology allows for rapid assay of all target organisms for which tests were developed under this project and SHP.005 to be run a single soil DNA sample and for that DNA can also used by for parallel research on soil communities structure and functional gene analysis.

Field application
Most of the newly developed tests were applied to field experiments in South Australia, NSW and Western Australia. They were applied to samples from field bioassays conducted by SARDI (this project and the lucerne establishment project under the National Action Plan for Dryland Salinity) and the MLA soil biology project run by CSIRO. In addition, the MASTER site (a long term lime x phosphorous grazing trial run by NSW DPI) and two EverGraze (developing perennial based grazing systems) sites (Albany and Wagga Wagga) were resampled. At the MASTER site, sampling was conducted in Winter, Spring and Autumn, with the latter two samplings being deep cores to validate the assessment of the target organisms down the profile.

For the existing tests, results were consistent with those obtained in the previous year, indicating a marked degree of stability in soil biology between the last 2 years and that the sampling and assessment methods are robust.

The application of the tests at different depths down the soil profile showed management (in this case liming treatments) could influence root development and the distribution of soil organisms to depth. This work highlights the value of the species-specific soil DNA assays for studying vertical distribution patterns and seasonal changes in soil. The data collected from the MASTER site could not be obtained in any other way.

Although some of the tests have not been fully calibrated, either because of technical constraints (e.g. AMFs) or because they are still under development, their application to field experiments provided evidence of the robustness of the test and the importance and/or properties of target organism in pasture soil.

The large and growing suite of tests represents some logistical challenges. However, the data shows strong consistency within sites and between seasons. This provides confidence to first assay

for broad range of targets to characterise the site and then focus on key organisms, to optimise the resources used in field research.

Pilot education and awareness package
This repeated the activities described in section 4.2.2 (SHP.018) where the pilot training presentation package and manual were revised, updated with new information and re-developed to support introduction of the DNA assays to both agricultural and natural resource management researchers and advisors

Workshops were conducted at Bendigo Vic. and Murray Bridge SA. These were attended by 35 scientists from State agricultural research and extension agencies, NRM Boards, the Murray Darling Basin Commission (SA) and some producers. Both production and NRM researcher and advisors were targeted to create awareness of the potential application of the molecular assays for soil/water borne organisms, function and plant roots and stimulate their interest in including the technology in future research projects in both production and NRM science projects. A key element was to stimulate interest in continued investment in further development and application of these technologies. The feedback from participants was positive and constructive with particular interest in the application to research around plant roots and terrestrial water quality. Suggestions for future developments of these technologies were captured to provide background for any future investment framework.

Where to next
It is recognised that development of the DNA assays has provided a substantial foundation to tackle research questions at a scale and pace not previously possible. To capitalise on this, further development of the technology is required. This should be aligned with research programs which aim to improve plant performance to assist the livestock and grains industries adapt to drought, climate change, rising input costs etc. We will continue to seek opportunities to develop and utilise the technology. Research Corporations should consider further development and use of the technology when setting research priorities and funding new programs.

Comments

The SARDI team have delivered well above expectations in terms of developing new molecular assays and, importantly, have been the research “hub” to which a number of other projects have been attached – eg SHP.017, SHP.002, SHP.004 and SHP.025 referred to above and which used or participated in molecular assay validation.

A particular highlight to emerge from these projects (SHP.005, 024 has been the assays for particular plants which can be used to study the dynamics of root growth and response to management, environment and soil borne organisms. An important feature is that the assays detect live cells in plants roots and so discriminate between active and redundant, structural components of roots as well as dead roots of the same species. They can also discriminate between plant species in mixed swards.

This outcome was not envisaged at the beginning of the Program, but has been a major advance in research capability to learn how roots behave in soils without destroying plants and endure the high labour and time costs of taking large soil samples and washing soil from roots during which process

a large proportion of fine roots, root hairs and live root cells are lost. Hence there is now a more precise, responsive and efficient technique now to follow roots in soils.

The outcome from this project has been to develop a suite of DNA based assays to efficiently assess within mixed sward pasture contexts and on a single DNA sample extracted from representative single soil samples:

- Important soil borne pathogens
- Important beneficial soil borne organisms
- Plant roots from a range of plant species
- Soil biological function potential

Another important achievement of this work has been to build a technology platform and a complementary delivery program for these new technologies directed at researchers and advisors to encourage the use of these assays in research and monitoring activities.

4.2.5 SHP.018 – Pasture Soil Biology Molecular Assays – Pilot Delivery

Authors: Dr IT Riley and Dr AC McKay - SARDI

Report Summary

This project was developed to support delivery of new DNA-based assays to pasture research groups for use as research tools to study soil biological constraints to production and sustainability.

SHP.005 project has been developing molecular assays for a range of soil organisms including pathogens and beneficial organisms and key pasture plant species. The latter are being developed to provide a DNA option to assess root growth in soil. Concurrently, Dr Steve Wakelin, CSIRO Division of Sustainable Ecosystems, has developed DNA assays for soil biological function and community structure. A number of assays from both groups have reached a stage of development where they can be evaluated in field trials, and help determine if they need further refinement.

The combination of these assays is expected to provide powerful new tools to study biological constraints to pasture production.

To engage pasture researchers and gain valuable field experience, four pasture trials were selected to evaluate the most advanced assays. These included recently established EverGraze sites at Wagga Wagga, NSW and Albany, WA, and two long established experiments including the MASTER site run by NSW DPI, Wagga Wagga and a grazing by phosphorus experiment run by CSIRO Plant Industry, Hall, ACT.

The results revealed significant treatment affects at each site. At the MASTER site a significant impact of lime was detected on levels of arbuscular mycorrhizal fungi (AMF) and several important plant pathogens including Take-all. While levels of some pathogens such as Pythium and *Rhizoctonia solani* AG2.2 were not affected by liming. This was correlated with sub clover soil DNA levels, which were also not affected by lime. Sub clover is a host of both pathogens. In the P X Grazing trial, the DNA assays detected differences in sub clover, *Pythium* and *Phytophthora*

clandestina levels between treatments. Treatments differences reflecting the different pasture compositions were also evident in the EverGraze sites.

Use of molecular assays to assess plant DNA levels is soil, is a novel approach to monitor root growth. Combining assays for different plants, pathogens and beneficial organisms should provide a better insight to biological constraints impacting on productivity and water use efficiency, and ultimately facilitate development of more efficient and robust pasture systems.

The CSIRO assays for key functional genes and community analysis also detected significant changes in abundance of functional genes and species composition between the different treatments, which included liming, grazing rates, P application and pasture composition.

A pilot training course and draft manual was developed to support introduction of the DNA assays. Workshops were conducted at Wagga Wagga NSW and Albany WA. These were attended by 30 scientists from State agricultural research agencies and universities. Their backgrounds included senior pasture researchers, visiting scientists, research students and some senior technical staff. The material was well received and stimulated considerable discussion on how this technology could be incorporated in the research programs. The feedback from participants was constructive and will contribute to refinement of future training activities, and priorities for development of future assays.

Engagement with pasture scientists through this project has further highlighted the value of the DNA approach to provide a practical method to study soil biological constraints as part of development of robust pasture management systems. There is strong interest in seeing the technology grow and applied and particular enthusiasm for using the plant assays to monitor root growth in soil.

Further development and promotion of DNA assays as research tools is warranted.

Comment

The projects described in this section were highly successful in demonstrating the potential power of the molecular assays for soil borne organisms, plant roots and soil biological function to discriminate between established treatment effects in a range of experiments.

In addition the assays for soil microbial function and community analysis indicated potential for use in research on the environmental impacts and greenhouse gas emissions from different grazing systems in response to management, plant species, climate and landscape differences.

The training for researchers was well received although it is clear that it must be on-going and with sufficient follow-up information in update sessions to convince researchers, who have given little thought to soil biology or considered it an area of investigation that is too complex, to embrace the power of these new research tools.

The potential of this technology to enhance and accelerate research into a wide range of soil biology issues and in-time offer producers with valuable diagnostic and monitoring tools for soil biological and plant root health is remarkable. For this to occur, more target specific molecular assays need to be developed and they all need suitable calibration and validation to optimise their application and interpretation across diverse environments and seasons and then to develop reliable management

responses from their interpretation. Patient, long-term investment in further technology development and applied research partnerships are required to realise the potential of this technology to help underpin, robust, resilient and productive pasture based agricultural production systems.

The combination of molecular soil biology assays developed for pasture systems with those developed for cropping systems offers a significant advance in the capacity to undertake soil biological research in mixed farming systems where successful transitions between crop and pasture phases are critical enterprise success and risk management in these systems.

As these molecular assays are developed they incrementally provide a powerful suite of research assets for use over the long-term in soil biology research across diverse research themes.

The technology also has application within natural resource management contexts and while attempts have been made to promote the technology to this sector, it is likely to take time to encourage investment from this sector.

A long term R&D program should be developed to ensure the technology is customised to address the current issues confronting the grazing industry such as development of more water efficient and drought tolerant pastures and pasture management systems in the context of adaptation to climate variability.

4.3 Soil Biology at the Root Surface – Strategic Basic Research

Two pieces of more fundamental research were commenced to better understand the dynamics on interactions between roots and the microbes at the root surface (rhizosphere).

4.3.1 SHP.007 – Pasture Plant Interactions with Soil Organisms in the Rhizosphere

Authors: Post Doc – Dr Y Cheng with Dr Alan Richardson – CSIRO, PI

The purpose of this project was to examine a hypothesis that factors which impact on plant stress or health can induce changes in the nature of biochemical exudates from plant roots into the rhizosphere and thereby influence the nature and balance of beneficial and pathogenic organisms in the rhizosphere that respond to these changing biochemical conditions. The objective was to begin to look for some fundamental plant physiological mechanisms associated with this phenomenon, if significant to plant health risk, as a basis to begin to develop plant and pasture management strategies which favour plant root health and function.

Report Summary

The objective was to undertake basic science to explore new opportunities for investigating the interaction of soil microorganisms in the rhizosphere of pasture plants. The project aimed to develop and apply new technologies to visualise and quantify the effects of soil microorganisms on plant growth, with a view to identifying and developing management strategies that optimize the contribution of microorganisms to the productivity and sustainable of pasture systems.

Although it is widely recognised that biological constraints may limit the productivity of Australian pastures, there is relatively poor understanding of the actual contribution of soil microorganisms to

the growth of pasture plants, which includes both pathogenic and beneficial microorganisms. However, the level of interest among producers in maintaining 'healthy' soils has increased significantly in recent years and there is widespread interest in better understanding the 'biology' of farming systems. Critical to this is greater understanding of how different microorganisms interact within the rhizosphere of plants and their subsequent effect on plant growth.

Major achievements of the project were:

- A survey of the literature showed that biological constraints are an important issue that can potentially reduce the productivity of Australian pastures and that microbial interactions in the rhizosphere may mitigate the severity of root diseases.

- The importance of disease on the growth of pasture plants was demonstrated in glasshouse experiments using intact cores of field soil. Whilst incidence of disease was highly variable, a number of potentially important root pathogens were identified. Controlled studies in sand culture showed that infection of roots of pasture plants by *Pythium* sp. significantly reduced root growth, whereas less consistent results were obtained in soil.

- New methods for the study of rhizosphere microorganisms were developed. This included a fluorescence-microscopy technique for direct visualisation of *Pythium* infection on pasture roots and procedures for 'tagging' different species of rhizobacteria with fluorescent proteins. Bacteria that inhibited the growth of *Pythium* in laboratory media were isolated from the rhizosphere of pasture plants.

- A system for the collection of root exudates from pasture plants was established for sand-grown plants and the C composition of the exudate was analysed by GC–MS. Defoliation of plants caused a significant change to the allocation of C to root growth and the quantity and composition of C in root exudates, which has important implication for root interactions with soil microorganisms. Attempts to isolate root exudates directly from soil-grown pasture plants were unsuccessful.

- The presence of various fungal (and oomycete) root pathogens and beneficial fungi in soil and on plant roots was investigated by quantitative DNA analysis (collaboration with SARDI). Although the presence of plant roots increased the DNA content of different fungal groups, no specific effects occurred in response to defoliation. Microbial DNA content of soil was also generally not affected by P status, although higher mycorrhizal content of roots was evident in low P soil, with a possible interaction between the presence of mycorrhizal fungi and reduced presence of *Pythium*. However, in all cases there was high variability in the fungal DNA content of soil which was exacerbated by the presence of plants. No clear relationship was identified between changes in microbial DNA content in soils with changes in the rhizosphere, and to the extent of fungal infection on plant roots.

Results from the project showed that DNA-based technologies hold considerable promise for investigating the behaviour of specific groups of microorganisms in the rhizosphere of plants. DNA-based techniques provide insight into the response of microorganisms to soil and plant treatments in ways that could not be achieved using more conventional techniques. The study also showed that GC–MS has considerable potential for quantifying the C composition of root exudates and to the

understanding of how plants respond to treatments that are indicative of management options for pastures.

Microbial DNA assays were successfully quantified groups of pathogenic and beneficial microorganisms, directly in bulk soil, rhizosphere soil and within plant roots. However, microbial DNA concentrations in soil were subject to high variability which was largely associated with intrinsic spatial variability in the distribution of the microbial species in field soil which were used as intact cores of soil. High intrinsic variability of different pathogens was further accentuated by the stimulatory presence of plant roots, which meant that it was difficult to interpret results from treatment effects that were imposed under glasshouse conditions. Whilst a number of measures were undertaken to limit this variability (i.e. increased replication, use of mixed and reconstituted soil cores rather than intact field cores, and controlled inoculation treatments), these also proved to be largely unsuccessful for root pathology studies in the glasshouse. In addition, it was evident from glasshouse studies that the presence of high DNA content of specific pathogens was not necessarily indicative of high incidence of root damage and/or clear evidence of root disease.

Further studies to correlate measured DNA content of specific pathogens in soil and incidence of disease on plants roots is required, as this relationship remains poor. Whilst it may be a consequence using glasshouse-grown plants, we are also aware of similar issues under field conditions. In addition to ensuring that the DNA probes used are representative of causative organisms, further investigation of the importance of environmental factors (e.g. soil type, climatic conditions, management practices, etc) that contribute to the 'outbreak' of root diseases is needed. As well as greater emphasis on use of field sites, this will require input from a plant pathologist. Future studies on the molecular ecology of soil-borne root pathogens, therefore, need to focus on field studies, with glasshouse studies limited to validation (e.g. pathogenicity tests in sand culture) and/or high-throughput germplasm screenings where artificially high inoculum levels can be used without compromising the outcome.

Improved understanding of how soil microorganisms interact with plant roots and respond to pasture management will, in the longer term, allow more informed decisions to be made in soil health and the productivity and sustainability of pasture systems. It is expected that such information will be readily available to growers and routinely used within the next 5–10 years.

Comment

This project represented a very challenging area of basic science but which, nevertheless, has great potential to underpin major advances in our knowledge of the impacts of management, including grazing, on rhizosphere biology and its influence on pasture plant productivity.

The project was criticised in the program review (Section 5) for limitations in the literature review in key areas and for a lack of focus resulting in the project following, and changing between, a number of lines of enquiry which hampered the project with respect of its original purpose. Adjustments were made after the review.

However, major achievements were made in developing techniques to pursue this area of research further. A critical challenge is the spatial variability associated with this work and which is constrained by physical limitations on replication in glasshouse experiments.

This area of research should, however, be pursued as a matter of priority and with an understanding that research investment must be patient due to challenges in complexity and valid experimental techniques. An understanding of the key mechanisms in root rhizosphere biology affecting plant health and productivity should lead to significant advances in the development of pasture plants and pasture management tactics to influence plant health and productivity.

4.3.2 SHP.015 – Responses to quorum sensing signals in pasture plants

Authors: Dr U Mathesius, ANU

This project commenced as a PhD but was found to be too high risk for the student when some early experimental technique challenges required resolution. The project was then restructured to support a technician for Dr Mathesius to allow for the risks associated with this quite basic science investigation.

The objective of this project was to establish whether there are any growth responses and improvements of plant-microbe interactions after treatment of pasture plants with quorum sensing signals (QSS) and soil bacteria. Plants and QSS-producing bacteria have co-evolved over a long time and the ability of plants to respond to QSS may enhance interaction with bacteria and other soil organisms. If plants perceive QSS, this could cause activation of defence responses against pathogens, change exudation of compounds used as nutrient or toxins against bacteria, or change the uptake of nutrient.

The project also aimed to test the effect of synthetic AHLs (QSS) on the growth of pasture plants, using model legumes of interest as they form symbiotic relationships with rhizobia. Perennial ryegrass is being used as a non-legume species because of its association with an endophytic fungus.

A second part of the project sought to focus on potential beneficial effects of quorum sensing signals of plant growth promoting bacteria on the protection of plants against soil fungal pathogens.

Report Summary
Part 1
In the initial phase of the project it was firmly established that plants have specific physiological responses to AHLs, that AHLs alter plant-microbe interactions, and that AHLs could be used to enhance plant growth in the field. The project was slightly modified in the last few months to concentrate more on root fungal pathogens, as these seem to be a major problem in pastures.

Conclusions so far are:

A systematic analysis of the effect of AHLs on plants grown in soil showed a significantly positive effect of C8-HSL treatment on plant growth in subclover grown in sterile soil. A similar result was found in non-sterile soil, but these results were not as significant. The result is interesting in particular because the C8-HSL which had a positive effect is produced by the *Rhizobium leguminosarum* symbiont of subclover, whereas AHLs produced by *Pseudomonas aeruginosa* had no effect. This indicates that clover recognizes the AHL produced by its symbiont and that this can enhance plant growth and nodulation specifically. Interestingly, this effect was not found for *M. truncatula*. Even though its symbiont *S. meliloti* also synthesizes some C8-HSL, its main AHLs are longer chained molecules. Pretreatment of seeds with

some or all of the AHLs produced by their rhizobial symbionts could be assessed in future as a treatment to improve nodulation rates in the soil.

The treatment with the C8-HSL also significantly enhanced seed germination, and this was strongly concentration dependent. This first suggests that AHLs from symbionts can affect plant development independent from nodulation, and second that there might be a receptor-based recognition of AHLs in plants (because of the concentration dependence). No eukaryotic AHL receptors are known. It would be extremely important and interesting to explore this possibility in future.

A further investigation of effects of AHL mutants of *P. aeruginosa* on plant growth in soil suggested neutral effects of the wild type on *M. truncatula* growth, and variable effects of the mutants. The effects of *P. aeruginosa* will be further investigated in the next stage of the project as a means to reduce rhizoctonia infection in the soil. Therefore, knowing the effects of *P. aeruginosa* on plant growth is a starting point to assess their effects in a tripartite system.

It was found that AHL treatment did not prevent the formation of root galls by the parasitic nematode *Meloidogyne javanica*. However, there were effects of AHL treatment on the number of galls and the root and shoot growth of subclover, suggesting that AHLs perception and response could interact with the infection process.

Also, a positive effect of AHL treatment on seed germination was found in soil containing *Rhizoctonia solani*. Our hypothesis is that this could be due to the stimulating effect of certain AHLs on seed germination alone, thus the seedlings could quickly establish themselves before the fungus infects. These results are encouraging for the next stage of the project in which the potential of AHLs and bacteria producing AHLs on suppression of fungal root pathogens will be further investigated.

Part 2

This project was initiated as a discovery project to investigate the potential of bacterial quorum sensing signals (QSS) as plant treatments to enhance pasture plant growth and health. It sought to establish a proof of concept for the effectiveness of QSS in improving plant growth and plant interactions with soil microbes.

QSS are chemical signals (acyl homoserine lactones) synthesised by most gram negative bacteria. Bacteria use these signals to regulate behaviours that require bacterial cooperation, for example when infecting a host. This has been demonstrated to be important for infection of plants with pathogenic bacteria (for example soft rot bacteria) as well as symbiotic bacteria, (for example rhizobia). Different bacterial species produce different chemical variations of QSS.

The background for the work was established by previous research showing that purified or synthetic QSS from bacteria can alter gene expression in plants when applied to roots (Mathesius et al., 2003). This suggested that plants can perceive these bacterial signals, and perhaps use them as cues to detect bacteria by their QSS ‘signature’ in the rhizosphere and to prepare for interactions with these bacteria, whether symbiotic or pathogenic. However, it had not previously been tested whether this ability to detect the signals and respond at a molecular level results in altered plant performance. The gene expression studies showed that genes important in plant defence, metabolism and hormone control were affected by QSS. This suggested that QSS could (i) alter plant defence responses and thus influence subsequent plant microbe interactions and (ii) influence plant hormone metabolism that could result in changes to plant growth. These hypotheses were

tested in selected pasture plant species: *Lolium perenne* (perennial ryegrass), *Trifolium subterraneum* (subclover) and *Medicago truncatula* (barrel medic). Under sterile and non-sterile conditions, these plants or seeds were exposed to synthetic QSS that are known to be synthesised by soil/rhizosphere bacteria. Their subsequent germination, growth, nodulation (where appropriate) and interaction with pathogenic soil microbes was assessed.

The project established a proof of concept that certain QSS can affect plant growth, germination and plant interaction with soil microbes.

First, application of certain QSS accelerated seed germination under sterile conditions in all three plant species. The effect depended on the concentration and structure of the QSS. Under non-sterile conditions, a smaller effect on germination was shown. However, this effect was transient and plant growth was no longer accelerated after one to three weeks of growth.

Second, application of QSS as seed dressings or as a regular leaf spray altered plant growth. In some cases, leaf and root growth (weight) was significantly increased by the application of QSS. This effect again depended on the plant species, the structure of the QSS and the growth conditions and suggests specific recognition of QSS by plants.

Third, pre-treatment of the two legumes, subclover and medic, with QSS influenced their ability to form nodules in symbiosis with rhizobia. Whereas QSS did not enhance nodulation under sterile growth conditions, nodulation could be enhanced in semi-sterile soil experiments. This also resulted in improved plant growth. The QSS synthesised by the specific rhizobial symbionts were most effective at enhancing nodulation, whereas other QSS were ineffective.

Fourth, the effect of QSS on the interaction of plants with fungal or oomycete pathogens were tested. Pathogens selected for this study were those that have been shown to limit pasture plant growth and included *Rhizoctonia solani* AG 2.1, *Phytophthora medicaginis* and root knot nematodes (*Meloidogyne javanica*). The hypothesis behind this was that treatment with QSS could affect general defence responses in the plant that could then prepare the plant for a subsequent attack by pathogens. The results showed no significant effect of QSS treatment on the development of root galls by root knot nematodes in medic, and a small reduction of gall numbers and plant growth in subclover. Both species were similarly infected by this pathogen. *Rhizoctonia solani* appeared to colonise both subclover and medic, but subclover showed no inhibition of growth in response to Rhizoctonia, whereas medic was significantly affected. Certain QSS enhanced growth of Rhizoctonia infected subclover roots. Certain QSS had a small beneficial effect on Rhizoctonia-infected medic when used as a regular leaf spray but not when used as a seed dressing. *Phytophthora medicaginis* severely infected medic and killed plants within 1-2 weeks. This was accompanied by production of a specific flavonoid in the roots that was used to monitor infection. Certain long chain QSS inhibited the accumulation of that flavonoid and alleviated early symptoms, but did not prevent the eventual devastating effect of *P. medicaginis*.

This project was designed to focus on the basic research on plant perception of QSS. It was only four years ago that the first reports emerged that indicted that plants can perceive and respond to QSS. Therefore, this research is still in a stage of discovery, with limited applications unless further research is carried out. To benefit from this research, QSS would need to be tested under field conditions with the local soil and pasture plants. The research done in this project could guide those tests by providing information about active QSS that could be tested, how they could be applied to

the plants and what outcomes might be expected. However, since this project found that plant responses to QSS depended strongly on the exact structure and concentration of the QSS, as well as on the growth condition and plant species studied, the final outcome of the QSS-plant interaction in the field is difficult to predict.

Farmers interested in testing QSS for potential to enhance their pasture plant growths could benefit from the results. Many QSS are commercially available and could be tested as seed dressing before pasture plants are sown into the soil. This might enhance seed germination and could potentially benefit plants by being able to outgrow pathogens present in the top soil layers. The QSS could also be further tested by seed companies or research and development organisations.

In addition, the observation that subclover was not affected by *Rhizoctonia solani* AG2.1 under the conditions used could be further investigated to determine the factors that could make subclover somewhat resistant. Since the application of certain QSS further enhanced growth of Rhizoctonia infected subclover, their potential in alleviating this important fungal pathogen disease could be further tested.

Comment

This project is true discovery research and has progressed very well under the model of providing technical assistance in a high risk area. The aspirational goal here is to discover novel ways to utilise specific signalling compounds, which may be systemic, to influence the plant root rhizosphere environment and thereby the nature of the organisms that colonise this zone with a view to stimulate improved plant growth and resistance or tolerance to root pathogens.

This work was conducted to a very high standard and efficiently.

While this area is complex, the findings of the above project provide a valuable foundation on which to build further work in this area and further investment is recommended. There is prospect for the technology to be developed where the artificial application of appropriate AHL's to plants may have a beneficial effect on plant health and performance.

To provide focus, a suggested research approach is to investigate the mechanistic differences between subclover and medic in their resistance/tolerance to rhizoctonia infection and the role selected AHL's in these differences. It may lead to the breeding of rhizoctonia resistant pasture plants and/or also the topical application (seed or leaf) of AHL's to induce a resistance response. This would be a major break through as rhizoctonia is a major soil borne pathogen of many agricultural plants and it is currently assumed that any plant-specific resistance/tolerance to this pathogen (and its bio-types) is weak.

5 Program Review

Professor John Irwin (University of Queensland – ex CEO, CRC Tropical Plant Pathology) and Dr Albert Rovira (Rovira and Associates – pioneer in agriculture soil biology, molecular assays, ex CEO of the CRC for Soil and Land Management) were engaged in 2006 to independently review the program part way through its progression.

Report Summary and Recommendations

Soil biology is an extremely challenging area of research, due to the complexity of the interactions between soil microbes, plants and the soil. Included in the category of soil microbes are beneficial organisms and root pathogens, with the most numerous soil microbes being bacteria. Plant type, soil type and soil management regime affect the microbial diversity of soil, which has implications for the level of disease suppressiveness of the soil, and plant health. To indicate the level of complexity of the biological interactions in soil, bacterial populations in soil top layers can go up to more than 10^9 cells per gram of soil, and most of these cells are generally unculturable.

Recent DNA-based methods for identification and detection of microbes in soil have offered the opportunity to gain new insights into and a greater understanding of soil biology, thus by-passing the limitations of cultivation-based isolation and identification methods for microbes.

The Soil Biology Program set out to:

- quantify losses due to pathogens using biocides at a range of sites
- develop DNA based methods for detection of known fungal, oomycete and nematode pathogens of pasture, and for known beneficials such as Arbuscular Mycorrhizal Fungi
- gain a greater understanding of the effect of pasture management on rhizosphere biology, and the potential to manipulate quorum sensing signals between bacteria and plants to enhance plant growth
- prepare a manual of extension of the DNA probe technology, firstly to researchers, then to industry

Root Disease Pathology Research

During the first 3 years of the Program, the researchers found the approach using biocides to quantify productivity losses was not successful. A field bioassay procedure was developed, and this bioassay, in conjunction with rapid and effective progress in developing the DNA probes, has provided a powerful new tool to assess the relative importance of the known root pathogens. We regard these developments as a highlight of the Program, and the researchers are to be commended for the cooperative collaboration and coordination shown in integrating field and molecular aspects of the work. Should this work demonstrate that *Phytophthora clandestina* is the most important known root pathogen of sub clover in Australia (refer SHP.016), then there is a high likelihood that over the shorter term, a DNA-based soil test, in conjunction with resistant varieties, would do much to achieve the 10 percent productivity increase sought by industry. This would require adoption of Recommendations 1 to 3 of this Review. Varieties of sub clover regarded as resistant to *P. clandestina* may either not be resistant to all races, or not be adapted to dryland pastures, the major target of this Program. However, on-going sub clover breeding programs

located in South Australia and Western Australia, could provide useful varieties if the work was afforded a high priority.

The above is contingent upon validation of the DNA technology, and we believe this work is being hampered by the lack of experienced, traditional plant pathology skills that can be applied to the project, particularly in NSW and Victoria. We have recommended that a plant pathologist with traditional and molecular skills be appointed to assist with the validation work (refer Recommendation 2). Also, further survey work is required, using traditional methodologies, to determine whether other root pathogens not deployed in the probe set, are contributing to significant productivity losses. *Aphanomyces eutiches*, another oomycete, could be in this category.

Rhizosphere Biology Research

This is an extremely difficult area of research, but significant breakthroughs in increasing pasture productivity will be underpinned by gaining a better understanding of the interactions between the soil biota, particularly bacteria, and important soil processes such as carbon turnover and nitrification. Much more focus is required in the research if worthwhile progress is to be achieved. We have indicated that the current Rhizosphere Project should focus on the effects of defoliation on exudation of organic compounds from roots, and that it link strongly with the work on Quorum Sensing Signals (QSS). The QSS work should concentrate on bacteria which have known biocontrol potential, and on others which stimulate plant growth, all of which will be influenced by root exudation, and thus the need for close interaction between the relevant researchers.

We feel that if the opportunity arises, there is potential to initiate new work on the impacts of pasture composition and grazing on the diversity of functional groups of microbiota (principally bacteria) involved in carbon turnover. This work is currently directed towards cropping systems in the Mallee region, and the expertise resides in CSIRO Adelaide.

If a pasture focused project was commenced in parallel with the current cropping work, then synergies should occur which would produce outcomes beneficial to both cropping and livestock industries.

Recommendations

The Soil Biology Program has two primary aims, viz. first to reduce losses by soil borne pathogens (principally fungi and oomycetes) and secondly to gain a greater understanding of soil microbes (identity and function) and soil microbiological processes, all of which are profoundly influenced by plant species, soil type and management. The soil biota (much of it unidentified) includes beneficial organisms such as Arbuscular Mycorrhizal Fungi (AMF), as well as fungi and bacteria which can influence plant productivity by processes such as biological control of root diseases and plant growth promotion.

The funded Program supports projects which address these two areas of soil biology and pathology in a modest but focused way. We make the following recommendations for future investments in this area, being generally satisfied that the aims of the Program so far have been met, and that significant industry outcomes can be achieved within the 2007 – 2010 period. We also strongly endorse the continued support of applied and strategic research, to enable further benefits to be delivered over the 2010-2013 timeframe. *The recommendations are relevant to all industries*

involved with pastures e.g. meat, wool and grains and have been made on the understanding that at least current levels of funding will be maintained for another 3 years.

Recommendation 1:

That funding be continued, at least at current levels, for the 2007-2010 period, with a targeted strategic and applied approach. It is anticipated that funding will be needed for an additional 3 year period (2010 – 2013) to provide maximum opportunity to deliver Program outputs to industry. We feel a 10 percent increase in the productivity of pastures in southern and Western Australia could be achieved by that time.

Recommendation 2:

The work on DNA probe development for the key root pathogens including *Phytophthora clandestina*, *Pythium irregulare*, *Rhizoctonia solani* AG 2 has progressed at an exceptional rate. The technology has been integrated with field bioassay sites established to monitor and quantify the impact of root diseases on sub clover, overall the most important temperate climate legume. The technology can be further developed to quantitatively assess levels of root damage caused by the different pathogens, and, along with conventional plant pathology, should assist in resolving the complex aetiology of sub clover root rot. A draft manual for application of this technology is in preparation, and it is possible that a DNA test useful for farmers could be developed by the end of the next three year funding cycle.

We recommend that sufficient funding be allocated to continue the validation of the DNA probe technology, along with the field bioassay work to assess impact over a range of sites in Victoria, New South Wales, South Australia and Western Australia, and that a plant pathologist with traditional and molecular skills in root diseases be appointed to assist with the validation work. To provide critical mass, we recommend the appointee be located in the SARDI Laboratories which are involved in the Program. This could be considered as a strategic investment in intellectual capital which will facilitate early industry adoption of the technology. For *P. clandestina* at least, effective management strategies in the form of resistant cultivars are available if the technology demonstrates significant losses due to this pathogen, which is currently regarded as the most important pathogen of sub clover (M. Barbetti, SHP.016 abstract and page 18). It is highly likely that additional breeding and or selection is needed, due to resistant varieties not being adapted to dryland situations, and due to the existence of a number of pathogenic races of this organism. Work should also continue on the quantification of AMFs from the different assay sites, with the ultimate aim of understanding their role in pasture productivity.

Recommendation 3:

It is essential that the DNA probe validation research continues to make optimum use of existing long term pasture management trials such as EverGraze (Albany and Wagga), the long term Hamilton Trial and the Master Site (Liming Trial).

Recommendation 4:

We also strongly recommend that strategically focused work in soil biology continue, to gain further understanding of the complex interactions between the plant, soil and soil biota. The two projects on strategic research, viz. quorum sensing signals (QSS) and rhizosphere biology, have been chosen because of their potential to increase pasture production in the long term. These are two important

research areas which will provide an understanding of the biology of the very complex soil environment under pastures.

We recommend that the QSS project focus more on beneficial and growth promoting bacteria which are now being used to reduce root diseases of cereals. It should be possible to develop cooperative work with microbiologists in ANU, CSIRO and SARDI who have been researching biocontrol organisms. This project should also develop closer links with the rhizosphere research at CSIRO (SHP.007).

We recommend that the research on the microbiology of the rhizosphere and on the release of organic compounds from roots be continued but with more focus; and that the emphasis be on the impact of defoliation (grazing) on the microbial population of the rhizosphere and on the release of organic compounds from roots. This project should capitalize on the sophisticated equipment in the department, but in a targeted and focussed way. Subterranean clover and phalaris should be used in this project in order to relate the results to field studies. We also strongly recommend that links should be made with the QSS project. Both projects are located in Canberra and there is the potential to generate significant synergies through closer interaction between the two research areas.

Recommendation 5:

We also recommend that new funds be made available to expand some of the research being conducted by Dr Gupta and Dr Wakelin, CSIRO Adelaide, on the impacts of cropping, pasture composition and grazing on the diversity of functional groups of microbiota involved in carbon turnover. The preliminary results obtained by Dr Gupta from the Mallee region have shown an effect of cropping and grazing of pasture on the diversity of soil bacteria involved in carbon turnover, and on soil health. This is cutting edge soil biology which could be used to describe the health of the soil under pastures subjected to different managements.

Consideration should also be given to supporting innovative research being conducted by Dr Marten Ryder, CSIRO Adelaide, on the development and validation of a bioassay for soil health. This test, which will be available in kit form for agronomists and farmers, will have a wide application in determining the biological health of pasture and cropping soils and will complement the research by Gupta and Wakelin.

Recommendation 6:

There is a shortage of trained plant pathologists relevant to this activity in Australia. We strongly recommend that funds be provided for at least two PhD scholarships in the field for pasture soil biology. There should be an opportunity to build these scholarships into the above recommended projects.

Prioritised Recommendations

These recommendations are listed in priority order for funding.

Priority 1:

Our highest priority recommendation is that the work on DNA probe development (including *Aphanomyces*), field bioassay work to assess impact and extension manual development continue to be funded, at least at current levels for another three years. This and subsequent downstream activities provides the best chance of generating a 10 percent increase in pasture productivity over the next decade, and not to fund it, at least at current levels, would jeopardise gaining any benefit from past investment in the Soil Biology Program.

Priority 2:

It is very important that the DNA probe work be validated using traditional plant pathology techniques of isolation and morphological identification of pathogens. Our next high priority recommendation is that an early career plant pathologist, with traditional and molecular skills, be appointed to support the Program, particularly in southern Australia.

Priority 3:

There is a need to train plant pathologists with traditional and molecular skills in pasture pathology, to maintain declining capacity in the area. We recommend that at least one PhD scholarship in this area be awarded.

Priority 4:

Our fourth priority for funding is the project on Quorum Sensing Signals. We regard this as the highest priority strategically focussed project.

Priority 5:

We give next priority for funding to the research on rhizosphere biology, but with greater focus than currently exhibited. The emphasis should be on the effects of defoliation (grazing) on exudates.

Priority 6:

The starting up of a new project on the impact of management on carbon dynamics in pasture soils is given Priority 6, but we consider this an important area of strategic research which could add to our understanding of soil processes which ultimately could be managed to increase productivity and sustainability of pastures.

6 Success in Achieving Objectives

The program review strongly endorsed:

- The overall strategic approach adopted to guide the Pasture Soil Biology Program over the longer term
- The program management
- The achievements of SARDI with the development of molecular assays as research tools
- The achievements of the program so far
- The placement of projects with the most capable researchers
- The introduction of specialist plant pathology expertise to complement the molecular assays development and their application to field studies for developing management options
- A continuation of the program to at least 2013 to provide enough time to develop an adequate understanding of the complexity of soil biology in pasture based systems to capitalise on the knowledge for practical solutions for producers.

6.1 Molecular Assays – Achieved

The SARDI team have made impressive progress in developing a wide range of molecular assays for important soil borne organisms affecting pastures as well as innovative tests for plant roots.

Assay	**Application**
Heterodera trifolii	Pasture
Meloidogyne javanica/incognita/arenaria	Hort/Past
Pythium clade F	Past/Crop/Hort
Pratylenchus penetrans	Past/Crop /Hort
Phytophthora clandestine	Pasture
Rhizoctonia solani* AG 2.1	Vegetables / Past
Rhizoctonia solani* AG 2.2	Vegetables /Past
***Lolium* spp**	Pasture Roots
Phalaris aquatica	Pasture Roots
Subterranean clover	Pasture Roots
Lucerne – *Medicago sativa*	Pasture Roots
Barley grass – *Hordeum leporinum and H. glaucum*	Weed roots
Silver grass – *Vulpia spp*	Weed roots
Arbuscular mycorrhiza fungi X 5**	Beneficial
***Trichoderma* X 2 tests**	Beneficial

While the initial emphasis was on soil borne root pathogens there have been significant advances to level not anticipated at the beginning of the program:

- Development of assays for mycorrhiza which will significantly enhance the research capability for a range of plant species for which associations with these beneficial fungi are essential for production, persistence and resilience
- Development of assays for plants which, when applied to roots, offers the potential to revolutionise research into the dynamics of root growth and response to a wide range of factors that affect plant growth and performance.

Hence there is now a suite of molecular assays for a wide range of pathogens, beneficial organisms and roots that can be applied to the same sample of extracted DNA from single soil samples which offer new and powerful research tools to accelerate the development of knowledge in pasture soil biology systems to underpin improved productivity and sustainability with a soft environmental foot print.

Due to lack of resources, special emphasis on molecular assays for soil biological function were not possible in the program. However, the use of these assays in assessment of samples collected from some experimental sites in SHP.018 revealed the potential for these to discriminate between management treatments and pasture species in terms of their impact on changes in soil microbial communities and soil biological function (particularly the N cycle).

Hence, in summary, this aspect of the program has achieved more than anticipated at the outset. To be able to run all these assays on the one DNA sample offers enormous enhanced research capability for soil biology in pasture systems. When linked with the assays that already exist for cropping systems, the capability to comprehensively study soil biology in a wide range of agricultural systems has been significantly advanced.

6.2 Validate Biological Constraints - Achieved

That there are significant constraints on at least sub-clover and lucerne plant germination and early growth due to pathogen induced root damage is now clear. The field bioassay techniques have demonstrated clear effects of root damage across a wide range of sites even in dry years.

An example from Dr Richard Simpson (SHP.017) in 2007 suggests that sub-clover was achieving only c. 60% of potential growth due to root damage.

Evidence was also found of root damage to pasture grasses.

Further evidence for constraints was provided by the Barbetti et al literature review.

6.3 Rhizosphere Interactions – Proof of Concept Achieved – Challenges Identified

The work on Quorum Sensing Signals has achieved objectives to demonstrate proof of principle that plant growth responses and positive interactions between plant roots and microbes were possible and opens up the prospect for the use of systemic bio-chemical signalling compounds to affect

pasture plant performance. The project established a proof of concept that certain QSS can affect plant growth, germination and plant interaction with soil microbes. However this was “discovery” research which has given some underpinning knowledge on which to begin to decipher the complexity of the interactions and how they might be applied. This area of research is promising but in its infancy.

The work on plant rhizosphere responses to plant stress and the interactions with rhizo-microbes was challenged to establish appropriate experimental techniques. It was demonstrated that defoliation influenced plant carbon metabolism in the C released to the rhizosphere and the nature of the compounds but while the results could not be used for fine quantification there was evidence of variation in the nature and quantity of different compounds released from roots due to defoliation and differences again between subclover and ryegrass. This may have important implications for root interactions with soil microorganisms.

No clear relationship between microbial pathogen DNA in soils with changes in the rhizosphere could be established with respect to the level of fungal infection on plant roots. Further research is needed to decipher the links between pathogen DNA in soils, the degree of root infection and the expression of disease.

6.4 Commence Delivery - Achieved

The process of delivery of the technologies from the Pasture Soil Biology Program was commenced with two series of workshops conducted over two years targeted at mainly researchers and advisors with emphasis on creating awareness of the new DNA assays and encouraging their use.

It is important to note that deployment of the molecular assays as diagnostic tools for producers and advisors is premature at this time. For such a service to be offered, it is important that there is sufficient understanding of the dynamic interactions between soil born organisms, pasture plant species, environment and management so that reliable management advice can be offered. Hence this program has focussed on initial delivery to researchers to help develop the knowledge base necessary to offer diagnostic services.

The assays are useful now as monitoring tools to observe changes even though interpretation of the effects may be speculative until the underpinning soil biology knowledge is developed.

There will be a continuing need for development and delivery of some basic soil biology education for researchers, advisors and producers to under pin delivery of the technologies to emerge from this Program.

7 Impact on Meat and Livestock Industry – now & in five years time

Evidence from the work on characterising soil biological constraints under pastures suggests that there is a significant opportunity cost in unrealised pasture productivity and stocking rates in at least the southern pasture systems in Australia.

The field bioassay work suggests that loss of sub-clover seedlings was typically in the range of c. 20-35% at most sites, although up to 90% at some sites. Ryegrass was affected by 10-45%.

Simulation modelling suggests that this represents an opportunity cost to a typical southern Australian sheep enterprise of 25-58% due to constrained autumn-winter productivity caused by root damage to pasture plants.

Simulation experiments (Richard Simpson – pers comm) for experimental sites on the southern NSW tablelands in 2005 using the GrassGro pasture systems model consistently over-estimated the autumn-winter pasture growth yields by 300-1200 kg DM/ha depending on the year being simulated. It was suggested that soil-borne diseases may be involved in the discrepancy. If so, there would be a significant affect on animal production due to biological constraints.

There has been no equivalent assessment of grazing systems in northern Australia.

This Program has verified the proposition that soil biological constraints on pasture (and therefore, livestock) productivity is significant. Hence the ***potential impact*** on the meat and livestock industries has been demonstrated.

It is important to note that unless further research investment is made to help develop the management practices that minimise soil biological constraints, then the potential gains to the meat and livestock industries will not be achieved and much of the investment so far, wasted.

This Program has made a start to develop the enabling knowledge and technologies which can underpin the development of reliable pasture and grazing management to overcome a significant proportion of the apparent biological constraints to pasture production.

The initial Program long-term objective to facilitate increased pasture productivity by 10% is achievable over the next 5 year period provided on-going investment is made in targeted research, development and delivery. The molecular assays for soil borne organisms and roots offer a way to greatly accelerate this research to a point where the technology can be used by producers and advisors. However, these assays need on-going development and need also to be validated and calibrated under diverse grazing systems in a complementary field research program aimed at devising management practices for adoption by producers. If this is not achieved, the potential impact on the livestock industries indicated in this program will not be realised.

8 Conclusions and Recommendations

The Pasture Soil Biology Program has made impressive progress since its inception in July 2003 with the clear establishment that detrimental soil biology factors can significantly limit pasture production (particularly early season) with concomitant limitations on animal production. While the extent and severity of these limitations may vary with pasture systems, soil type, climate, landscape, management etc, there is now sufficient evidence to warrant further investment in pasture soil biology to enhance the water-use-efficiency of pastures and optimise productivity and health of livestock production systems.

There have been high levels of achievement in developing molecular assays as research tools for soil borne organisms, roots and soil biological function. This offers the opportunity to dramatically increase the research capability in soil biology of pasture and accelerate the advance of knowledge needed to develop optimising management practices for soil biological health in very complex systems over that possible with traditional methods of investigation.

There have also been impressive achievements in the basic, discovery science area of quorum sensing signals with the future prospects that application of systemic forms of signalling chemicals to plants can enhance plant performance by stimulating growth or systemic resistance to root pathogens. These studies also offer the potential to identify inheritable traits which may be used to develop breeding objectives and/or markers in plant improvement research.

The Program Review strongly endorsed the over-all strategy, the planned timeframe, the Program management and the emphasis given so far to the suite of projects embodied in the program. The reviewers strongly recommended that the Program continue. Their emphasis was on maintaining research into production limitations and did not consider any environmental aspects.

During the course of the Program so far, there has been a strong increase in concerns about environmental impacts of agricultural systems and with adaptation to climate change.

This Program has obtained evidence that the molecular assays for soil biological function can be applied to agricultural systems to further investigate the greenhouse emissions from pasture systems as well as provide a balanced focus on reducing production constraints induced by deleterious soil biological influences.

As per the original strategy, the Pasture Soil Biology Program has created new research tools and knowledge to better enable future research into understanding the dynamics of soil biology in Australian pasture and pasture-crop based systems so that management practices can be developed that favour soil biological health and underpin a significant increase in production and profit potential for producers.

8.1 Recommendations

8.1.1 Program Continuation

That the Pasture Soil Biology Program continue consistent with the existing, endorsed, over arching strategy with adequate resources to achieve the aspirational goal of at least 10% increased pasture production.

That the next investment phase be over 5 years with a longer term view to continue past this time to further research into the most beneficial aspects of soil biology of benefit to producers.

8.1.2 Parallel, Linked Production and Environment Research Themes

That the next phase of the Pasture Soil Biology Program embrace two parallel research themes:

- **Production** – pursue the aspirational target of increasing pasture productivity by 10%
- **Adaptation** – place some special emphasis on the challenges producers face in adapting pasture systems to climate variability, Also in light of producers needing to adapt to a possible

Emissions Trading Scheme being implemented for agriculture, place some emphasis on strategic elements of carbon sequestration and cycling in soils and plants and perhaps also some elements on green house gas emissions/control in pasture systems where believed significant.

In the suggested future Pasture Soil Biology Program, these two themes can be pursued together and at the same experimental sites as they will be linked. For instance, to increase pasture productivity will increase carbon fixation to contribute to the soil C pool; to match N supply to plant demand should help reduce leakage of nitrous oxide as greenhouse emissions and leaching of nitrate which increases soil acidity; molecular assays for plant roots will help understand plant adaptation strategies needed to successfully cope with climate variability.

8.1.3 Extension to Northern Australia - Knowledge & Opportunity Audit

That the Program be extended to relevant pasture systems in northern Australia to the extent that available funds will allow.

All the work so far has emphasised pasture systems across southern Australia whereas the red meat industries are well represented in the northern agricultural zones. More effort needs to be directed here for best over-all impact on these industries.

As a preface to work in northern Australia, a Knowledge and Opportunity Audit should be commissioned with special emphasis on identifying knowledge gaps appropriate to northern pasture/grazing systems.

8.1.4 Recommended Research and Resources for Future Pasture Soil Biology Research.

The recommendations from the Program Review are largely endorsed and the following areas of research for Phase 2 of the Pasture Soil Biology Program are recommended for consideration in future planning:

- **Ecology & Management of Root Damage in Pastures**
    - Continue field bioassays across more sites using the DNA assays to identify causal organism and also help calibrate the level of pathogens that represent risk of plant yield loss. This will be important to developing the DNA assays and diagnostic and monitoring tools for producers in aiding management decisions.
- **Molecular Assays as Research Tools – Production**
    - Continue development and validation of assays for organisms and plant roots important in pasture and pasture/crop systems – correlate with factors affecting production and environmental impacts
    - Evaluate assays for general soil biology parameters which relate to soil health
    - Continue to educate and engage with researchers on their use in research and monitoring

- **Nitrogen and Carbon Cycles in Pasture Soils – Production and Environment**
    - Use a combination of molecular microbial, soil function and chemical analytical techniques to assess pasture management, pasture type, soil type and season on:
        - The N and C cycles with special emphasis on greenhouse gas emissions, systems transformations, leakage and storage.
        - Fungi and bacteria microbial community diversity and function
    - Link with the effort on production and interactions with other organisms.
    - Place special emphasis on micro-ecological studies of carbon turnover, microbial community analysis and soil health in relation to pasture systems and pasture-cropping systems. Objective to develop basis for soil health benchmarks.
- **Pasture Plant Pathologist – Soil Biologist**
    - Engage a early career pasture pathologist to develop skills in traditional and molecular aspects of soil pathogens and beneficial organisms in soil and also develop skills in general pasture soil biology
    - This position should offer a significant career prospect and should be seen as a key plank in developing research capacity in this area for then next 10-15 years.
- **Lucerne establishment and productivity – Mallee and contrasting areas**
    - Resolve the risks of establishing Lucerne phases in cropping systems which recent surveys have shown to be affected by soil borne pathogens affecting establishment, survival and productivity.
    - Place some emphasis on management to optimise crop-pasture-crop transitions in mixed farming systems.
- **Interactions – Plant Roots and Soil Microbes in the Rhizosphere**
    - Continue basic research into understanding interactions at the root surface with clearly defined researchable questions of importance to plant productivity, disease resistance/tolerance and interactions with beneficial soil organisms.
- **Quorum Sensing Signals (QSS)**
    - Continue basic science investigating the signalling between plant roots ad soil microbes with a view to identify systemic signalling chemicals which can benefit plant performance and resilience.
- **Mechanisms in plants of resistance and tolerance to soil borne pathogens**
    - Strategic basic science to underpin breeding programs for these traits and development of disease mitigation management practices.
    - Include interactions with mineral nutrition – two key elements that constrain root growth – pathogens and nutrients
- **Root Growth in Soils**
    - Use the molecular assays for plant roots to study how plant roots penetrate hostile soils
    - Link to plant improvement programs to evaluate emerging varieties for better root penetration and survival in these soils – feedback to breeders on important traits.
    - Link to research on adaptation to climate variability and change – successful root growth and function will be a critical success factor for adapted plants
- **Beneficial Endophytes to Protect Pasture Plants from Soil Borne Disease**
    - Identify systemic endophytes as seed treatments for pasture seed to confer early vigour and resistance/tolerance to soil borne pathogens
    - Link with the QSS research

- **Soil Ameliorants – Impacts on Soil Biota**
    - Evaluate conventional and unconventional soil ameliorants (eg lime, charcoal, “organic” lobby recommendations etc) on soil biota as measured with the molecular assays.
    - Engage with producer groups to validate claims of soil ameliorants
- **Researcher Outreach – Support for Molecular Assays**
    - Provide a pool of funds for researchers of current projects to access to begin using the molecular assays within their project for which existing budgets are prohibitive.
    - Use the fund to leverage engagement by researchers with the technology.
- **Producer Engagement**
    - Design and implement small projects in partnership with producers with specific topics of interest in pasture soil biology.
    - Utilise the interests of producers in soil biology to stimulate adoption of wide ranging best practice advances in grazing systems while using the molecular assays to monitor effects on key soil biological parameters.

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10 Appendices

10.1 Field Bioassays to Assess Soil Borne Biological Constraints to Pastures

Widespread and substantial root damage on subterranean clover in autumn-winter pastures

Richard Simpson, Alan Richardson (CSIRO Plant Industry, Canberra); Ian Riley, Alan McKay, Ross Ballard, Suzanne McKay (SARDI, Adelaide); Tiernan O'Rourke, Martin Barbetti, Krishnapillai Sivasithamparam, Hua Li (University of Western Australia, Perth).

Why the work was done

Numerous studies report pasture growth responses to fungicides, nematicides and/or fumigation treatments suggesting that some aspects of soil biology potentially constrain pasture production. However, early work in the MLA-AWI-GRDC Pasture Soil Biology Program demonstrated the difficulties of using biocide treatments to explore soil biology constraints. Biocides are often only partially effective against the target organisms, the efficacy of soil drenches was affected by soil type and/or the biocides were phytotoxic for plant growth.

An alternative "bioassay" approach was devised to progress the assessment of potential for soil biology constraints in pastures.

Methods

The likely presence of root rot and damping-off pathogens was detected by sowing a susceptible cultivar of subterranean clover (cv. Woogenellup) about a month after the break of season in 2006, at 11 pasture sites/treatments in NSW, 4 in SA, 3 in WA. Lucerne (SARDI 10) was the test species at 4 sites in the SA Mallee (Fig. 1).

The top 0.5-1cm of soil was removed using a sharpened spade (Fig. 2). A shallow, 1m-long furrow was formed by pressing the edge of a star-picket into the soil. (Fig. 3). One hundred viable seeds were distributed along the furrow and covered lightly with soil (Fig. 4).

Seedlings were counted after: emergence (phase 1) and establishment (phase 2) (Fig. 5). The incidence and severity of root rot (damage) symptoms was recorded on roots of all surviving plants at the final count. Annual ryegrass (cv. Safeguard) was sown for comparison at all sites but was only assessed for emergence and establishment.

Figure 1.

Figure 5.

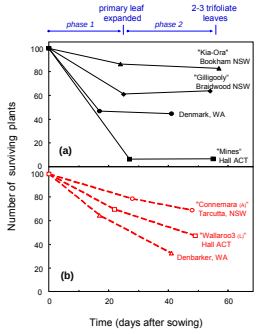
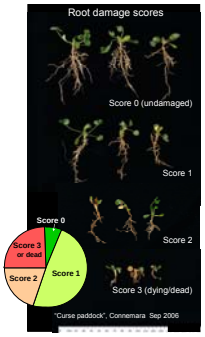


Figure 6.



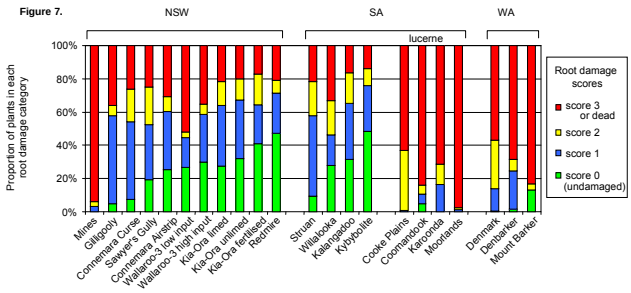
Results

All sites in the mallee were severely drought affected limiting the interpretation of their results. Consequently, only passing reference is made in this report to sites sown to lucerne. All but three of the subterranean clover sites had reasonable soil moisture conditions for germination and emergence. One (Mines, NSW) was irrigated until rain occurred; the others (Willalooka and Kybybolite, SA) emerged late when rain occurred. Data from the unwatered sites was not included in the analysis of seedling emergence.

Failure of seedlings to emerge: In moist soil conditions, seedling counts after emergence were expected to indicate losses due to "damping off" (Fig 5a). At 8 of 13 subclover sites in this category, equivalent proportions of clover and ryegrass (9-52%) failed to emerge. At other sites, more of one species failed. In the worst case, 93% of clover failed to emerge (Figs. 5a and 7).

Post-emergence clover losses: Loss of clover plants after emergence was considered likely to reflect the impacts of "root rot". On average, post-emergence losses were about 9%. However, the range was very wide (0-32%). Post-emergence loss of lucerne at one site was also estimated to be substantial (~40%).

Figure 7.



Root damage: Damage to subterranean clover roots was scored using a modification of the disease scoring system reported by Wong *et al*. (1984; AJAR **35**, 675) (e.g. Fig. 6). Root damage to Woogenellup subclover was extensive at all sites (Fig. 7). It was clear that root damage had impacted adversely on shoot yield (e.g. Fig. 6) but this was not quantified.

No site had more than 50% of plants germinating and establishing with undamaged roots. At a majority of sites <30% of plants were able to establish with undamaged roots.

Conclusion: Root damage on subclover in autumn-winter was widespread and substantial and was associated with significant seedling losses. Extensive root damage on the plants that manage to establish is likely to limit pasture growth rate at the key time of the year when pasture yield limits stocking rate.

10.2 Soil Microbiology Associated with Sub-clover Root Damage

Soil microbiology profiles associated with root damage in subterranean clover pastures

Ian Riley, Alan McKay, Ross Ballard, Suzanne McKay (SARDI, Adelaide); Richard Simpson, Alan Richardson (CSIRO Plant Industry, Canberra); Tiernan O'Rourke, Martin Barbetti, Krishnapillai Sivasithamparam, Hua Li (University of Western Australia, Perth).

Why the work was done

There are numerous reports that pasture growth can respond to application of fungicides, nematicides and/or fumigation treatments indicating that some aspects of soil biology may potentially constrain pasture production. A recent study across southern Australia as part of the MLA-AWI-GRDC Pasture Soil Biology Program has confirmed that root damage on subclover in autumn-winter is indeed substantial. Seedling losses during germination and establishment were significant and root damage on surviving plants was sufficiently severe to be likely to limit pasture growth at this key time of the year when pasture yield limits stocking rate.

New DNA probes for common root pathogens, some beneficial soil fungi and nematodes relevant to subterranean clover pastures were employed to establish the prevalence of selected soil micro-organisms at the sites where root damage on subclover was being assessed.

Methods

The likely presence of root rot and damping-off pathogens was detected by a plant bioassay using subterranean clover (cv. Woogenellup) about a month after the break of season in 2006, at 11 pasture sites/treatments in NSW, 4 in SA, 3 in WA. Lucerne (SARDI 10) was the test species at 4 sites in the SA Mallee (Fig. 1 and companion poster).

DNA probes for major plant types, common root pathogens, some beneficial soil fungi and nematodes relevant to subterranean clover pastures (Table 1) were used to probe composite soil samples (multiple 25 mm diam x 100 cm depth cores = ~500 g soil/replicate) taken from within, or adjacent to the plant bioassay area.

Table 1. Plant species, common root pathogens, nematodes and beneficial fungi for which DNA probes were available.

Plants	*Lolium/Fescue* spp., *Trifolium subterraneum* and *Phalaris aquatica*
Fungal pathogens	*Bipolaris sorokinana* (Common root rot), *Gaeumannomyces graminis* var. *tritici* and var *avenae* (Take-all), *Fusarium culmorum/graminearum*, *Fusarium pseudograminearum* (two types), *Mycospaerella pinodes*/*Phoma medicaginis* var. *pinodes* (black spot complex), *Phoma* sp (associated with Black Spot) and *Rhizoctonia solani* (AG2.1, AG2.2, AG4 and AG8)
Oomycete pathogens	*Phytophthora clandestina* and *Pythium* Clade F (after Levesque and de Cock, 2004)
Nematode parasites	*Ditylenchus dipsaci, Heterodera avenae*, *Meloidogyne javanica/incognita/arenaria*, *Pratylenchus neglectus, P. penetrans, P. thornei*
Arbuscular mycorrhizal fungi	AMF groups a, b, c, d and e

Results

The DNA-probe results revealed substantial differences in the plant and microbial profiles of sites and regions as illustrated by Figure 2 which shows the occurrence of clover pathogens at all of the subterranean clover sites.

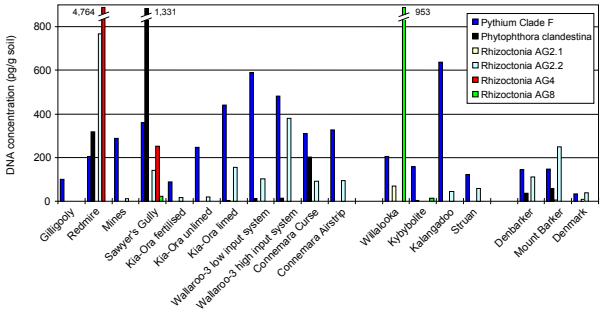


Figure 2. Concentrations of selected soil-borne pathogen DNA in soil under all of the subterranean clover pastures examined illustrating the diversity of the oomycete and fungal profiles of the sites. Note that only one pathogen may occur at a site (e.g. Gillygooly, NSW) while up to 5 (of the 6 pathogens for which DNA-probes were available) occurred at Sawyer's Gully, NSW. Paddocks on the same farm may have different pathogen profiles (cf. Curse and Connemara Airstrip paddocks). Some pathogen groups (e.g. *Pythium*-clade F) were very widely distributed, whilst others (e.g. *Phytophthora clandestine*, *Rhizoctonia* AG4 or *Rhizoctonia* AG8) occurred at discrete locations.

Of the oomycete pathogens, *Pythyium* clade F was the most common and was found at every site tested. *Phytophthora clandestina* was only detected in significant concentrations at three sites in NSW and two in WA. *Rhizoctonia solani* groups were common. Patterns of distribution AG2.1 and AG2.2 appeared to be distinct although they also occasionally occurred together. AG4 only occurred in significant concentrations at one NSW site and AG8 only at the Mallee sites or the most northerly of the SA sites. Phoma (black spot) occurred in moderate concentrations in the southeast of SA and was also present in WA. Some of the pathogenic fungi probes target cereal/grass pathogens. *Gaeumannomyces graminis* var *tritici* was common especially at sites with phalaris and probably other sites with moderate to high grass components. *Biploris* (common root rot) was common in the SA Mallee sites and at sites in NSW and WA but in all cases DNA concentrations declined in concentration. Detection of *Fusarium* species was either low or sporadic.

Mycorrhizal fungi (AMF) occurred at all sites at varying concentrations and combinations of clades. There was a range in clade diversity and abundance between sites and regions.

Overall nematode detection by DNA was low. Plant-feeding nematodes are almost certainly important in pastures, so it is likely that the results indicate that an alternative set of nematode tests are needed.

Conclusion: Use of DNA probes for disease organisms in pasture soils is in its infancy, but this study illustrated the ability of the probes to rapidly quantify the pathogenic or beneficial microbial profile of a site. It revealed how some pathogen groups were ubiquitous whilst the distribution of other organisms was discrete. It was evident that DNA assays provide a means to examine diversity and abundance of organisms (e.g. AMF) in pasture ecosystems that would otherwise be near impossible to study.

Root damage constrains autumn-winter pasture yield and farm productivity

ichard Simpson[1], Alan Richardson[1], Adam Stefanski[1], Ian Riley[2] and Alan McKay[2]

Introduction

Root damage on subterranean clover as a result of root-rot pathogens has been described variously as a substantial problem (yield reductions of >70%; Barbetti et al. 2006) or as an intermittent problem which can be severe in certain years (Murray et al. 1993). It is likely that the true cost of root damage is not fully appreciated because sub-lethal damage to roots is hidden to casual observation.

Method

Bioassays were established at 4 NSW sites about 4 weeks after the break of season by removing the top 0.5-1 cm of soil and planting 100 surface-sterilised seeds of subterranean clover (*Trifolium subterraneum* cv. Woogenellup) or annual ryegrass (*Lolium rigidum x multiflorum*). Seedlings were later excavated and the severity of root damage assessed, shoot DM determined and roots assayed for pathogen DNA.

Results

Occurrence and severity of root damage

Substantial numbers of subterranean clover seedlings failed to establish or had significant root damage in 2006 and 2007 (Fig. 1).

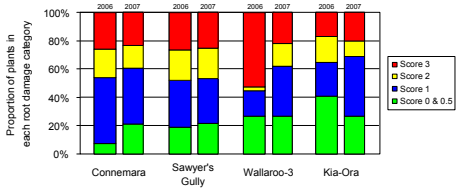


Figure 1. Proportions of subterranean clover (cv. Woogenellup) in each root damage category at the 2-3 leaf stage: 0 = undamaged, 0.5 = secondary lateral roots damaged and/or tap root brown, 1 = primary lateral roots damaged, 2 = tap root damaged, 3 = severely damaged roots leading to plant death, or seedling failed to emerge.

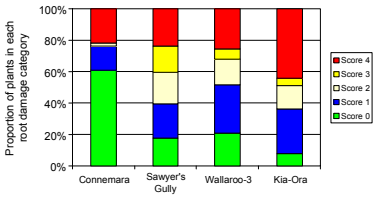


Figure 2. Proportions of annual ryegrass (cv. Safeguard) in each root damage category at the 2-leaf stage: 0 = undamaged, 1 = brown tips or a few brown seminal roots, 2 = all seminal roots brown to black, 3 = seminal roots absent, 4 = all roots brown to black or rotted, or seedling failed to emerge.

Grass bioassays in 2007 demonstrated that damage was also prevalent on grass roots, but a much wider range in outcomes was observed between the sites (Fig. 2).

Impact of root damage on autumn-winter growth

Increased severity of root damage was associated with reduced plant yield (Figs 3a,b).

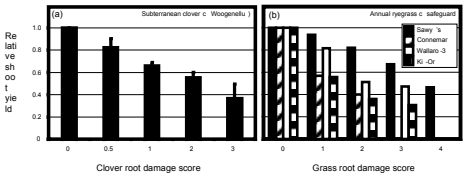


Figure 3. Relative yield of (a) sub clover (mean±SD 4 NSW sites) and (b) annual ryegrass (each site shown separately due to differences in response) for plants in each root damage category.

Predicted impact of root damage on net farm income

Calculations based on the occurrence of root damage and its impacts on autumn-winter yield at each site indicated that pastures were achieving between 63-82% of their potential yield. Simulations using AusFarm (GRAZPLAN pasture and animal models; Freer et al. 1997; Moore et al. 1997) suggest that root damage that constrains autumn-winter pasture growth is likely to constrain net farm income (Figure 4).

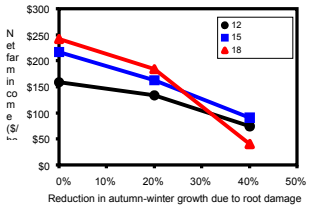


Figure 4. Predicted impact of root damage on net farm income for a Merino wether enterprise at Canberra. Simulation period 1970-2002.

Conclusion

Seedling losses and damage to roots of clover and grass were substantial. Autumn-winter plant growth was negatively related to the extent to which roots were damaged. 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 normally expected when stocking rates are lifted.

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10.4 Tip and Tool (draft) – Biassay Protocol for Assessing Root Damage on Subterranean Clover during Autumn-Winter

Aim: to estimate the prevalence/potential of root damage (disease) on subterranean clover during germination, establishment, and early growth in autumn-winter using a field bioassay.

Method:

Summary: the potential for root damage is detected by sowing Woogenellup (a subterranean clover cultivar susceptible to root rot and damping-off pathogens) with minimal disturbance into pasture sites. Seedlings are checked for (1) emergence, (2) establishment, and (3) incidence and severity of root damage symptoms. The bioassay method is based on Wong et al. (1985: *Aust. J. Exp. Agric*. **25**, 574-9) who were examining fungal root rots on subterranean clover.

Useful base measurements:

- Soil fertility: a single combined sample of **25** soil cores (0-10 cm depth) collected across the area to be used for the bioassay. Soil to be spread thinly on paper and air dried or oven dried at 40^{o}C prior to analysis. Do not microwave. Analyse for: pH(water), pH($CaCl_2$); Sulphate sulphur(KCl_{40}), Phosphorus (Colwell), Phosphorus (Olsen), Potassium, Calcium, Magnesium, Sodium, Aluminium, Chloride, EC, Cation exchange capacity, Phosphorus buffer index(Colwell), Total C.
- Botanical composition of pasture. Identification of main grasses, legumes and forbs to species level.
- Site mean annual rainfall, and weather and soil conditions during the assay
- Latitude, longitude, altitude (easily done by GPS)
- Recent management history (last 5-10 years)

Test species: *Trifolium subterraneum* cv. Woogenellup is used as a test plant for sub clover pasture sites because it has general susceptibility to root pathogens. Seed can be very variable in size and should be graded by sieving to obtain a uniform seed batch, about 2.2-2.8 mm in diameter (ie. mesh#6 - mesh#7; 0.09 - 0.11 inch).

Lucerne and grass species can also be used as a test plant. However, root damage scores for grasses will follow a different protocol to that described here.

Sowing:

1. Prepare lots of 100 germinable seeds per replicate bioassay (i.e. seed numbers are adjusted according to germination test to ensure correct number of germinable seed is sown). Prior to planting, seeds are surface sterilised by washing in 70% ethanol for 30 seconds and dried.

   Approximately 1 month after the break of season at each site prepare **EIGHT** replicate strips by scraping off the top 0.5-1 cm of soil from rows 1 m long x ~0.25 m wide using a flat, sharpened spade or trenching shovel (Fig. 1).

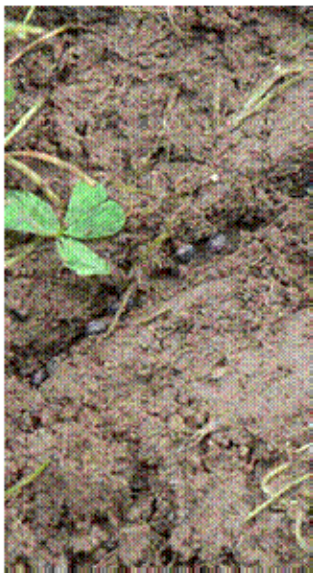
   Notes: Soil removal to this depth removes most other sub clover seed and reduces potential for interference from weeds. Extra rows may be included for "positive" controls if this can be accommodated. However, controls using fungicides or soil treatments may not be totally effective. Usually, some plants with relatively undamaged roots will exist at each site and after root damage is scored, these plants can be used as an indication of what plant growth might be possible in the absence of root damage. EIGHT replicate rows are sown in each treatment within each paddock to cover the intrinsic variability of root pathogens.

2. Press the edge of a clean, 1 m length of star-picket into the soil to form a furrow (~4 mm wide x 5 mm deep), sow seeds evenly along the row and cover by brushing soil over seed, tamp down and water gently if necessary (Figs 2 and 3).

Figure 1. Scrape top 0.5-1 cm of soil with flat, sharpened spade.

Figure 2. Press star-picket to form furrow into which seeds are sown.

Figure 3. Distribute seeds along furrow and cover lightly with so



It is necessary to protect the seedlings from red-legged earth mites by spraying the surrounding the area at sowing with bifenthrin (Talstar) at 100 ml/ha (as shown in Fig. 4). Insecticide should be applied as per the directions on the label. This product has residual effects and a 4-week with-holding period and may give up to 6-weeks protection. It is thought to be safe for spraying over the furrow in which the seeds have been sown. Other products may not afford equal protection and may not be suitable for sowing over the furrow (especially if systemic in action), or may have very long with-holding periods and can therefore be problematic for the farmer collaborator. Discuss these issues with your collaborator before starting.

3. In some locations protection from grazing by rabbits, kangaroos and other animals is necessary and can be achieved by using exclosures made of foot netting or similar material (Fig. 5).

Experimental layout: At each site, bioassay rows can be laid out as illustrated below. The area is not intended to reflect necessarily the whole of any particular farm paddock, but should reflect a site and cover the intrinsic variability of root rot occurrence. Rows may need to be marked with a peg so they can be found once pasture growth has got underway.

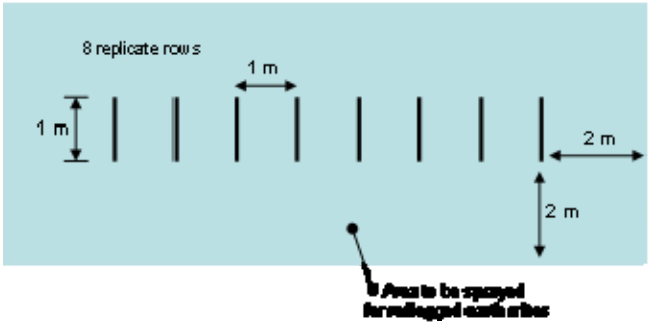


Figure 4. Layout for replicate bioassay rows and dimensions of buffer to be sprayed for redlegged earth mite control.

Figure 5. Exclosures being used to prevent grazing of test plants.

Monitoring:

4. at the primary leaf stage (about 2-4 weeks depending on climate and time of year): record number of plants that have emerged in each replicate. Provided soil moisture conditions are conducive for germination, plant losses at this stage are considered likely to reflect loss due to fungal "damping-off".

5. at the 2-3 leaf stage (about 4-8 weeks after sowing depending on climate and time of year):
   (i) Record number of plants established (plant losses at this time are considered likely to be due to root rots provided soil condition for establishment have otherwise been good),
   (ii) Dig up plants from the rows maintaining the replicate groups of plants; keep cool whilst transporting,
   (iii) Wash the roots and score for root damage severity on tap and lateral roots.

Notes: Washing roots to be scored for damage is labour and time consuming. Plants may be kept for some time at 4^{o}C in the soil clods that have been dug from each bioassay row. It is necessary to keep clods intact and access to considerable 4^{o}C space is needed. Once washed, roots may only be kept at 4^{o}C for up to a week provided they have been blotted dry and kept in closed plastic bags. They cannot be reliably scored after this period as they degenerate.

Root disease scoring:

This is a modification of the scoring procedure of Wong et al. 1984, *Aust. J. Agric. Res*. 35, 675-84).

Washed roots (intact seedlings) are floated in a shallow tray (white plastic meat trays [300 x 450 x 50 mm] are ideal) and sorted quickly into the 4 main groups (i.e. 0 – 3) and counted using the following simplified ratings scheme. Note: score 3 roots are on plants judged to be about to die; plants that have reached this stage previously will not be present for scoring! In some studies, it has been found useful to identify a subgroup in the relatively undamaged root category (designated score 0.5, see also Fig. 6).

Rating	Description
0	tap and lateral roots healthy and not discoloured
[*0.5*	*primary laterals present, but secondary laterals stunted or absent*]
1	whole tap root light brown to brown, primary lateral roots stunted or dying (brown)
2	tap root stunted and brown to black, discrete lesions may be present
3	whole tap root rotted off, or seedling is dead.

Calculating root damage indices:
Average root disease indices (%), based on the disease ratings for surviving and dead seedlings is then calculated using the method described by McKinney (1923, *J. Agric. Res*. 26, 195-218).

$$\text{Root damage index (damping off and root rots)} = \frac{\text{Sum of all numerical ratings x 100}}{\text{Number of germinable seeds x 3}}$$

$$\text{Root damage index (root rots)} = \frac{\text{Sum of all numerical ratings x 100}}{\text{Number of plants surviving the first assessment x 3}}$$

Sample calculation - If 35% of seedlings die before the first assessment and plant growth conditions have been moist and good for germination, it is likely that losses are due to "damping off" diseases.

Thereafter, if plant growth conditions remain good but a further 15% of seedlings are lost, they have probably died due to "root rotting" diseases. If then at the final assessment and harvest, the results of scoring are: 20% survive but with a root disease rating of 1; 15% survive but with a root disease rating of 2; 5% survive but with a root disease rating of 3 and 10% are unaffected:

$$\text{Root disease index (root rots)} = \frac{[(20*1) + (15*2) + ([15 \text{ dead} + 5 \text{ surviving}]* 3) + (10*0)] *100}{(20+15+[15+5]+10) * 3} = 56\%$$

The denominator represents the worst case scenario for root rot damage: i.e. all plants that survive the first assessment are counted and multiplied by a score of 3.

Estimating the impacts of root damage on plant growth:
Once plants have been sorted into their root damage categories and counted, shoots are removed from each group of plants by cutting through the base of the stems. The shoots are dried at 70^{o}C, weighed and shoot mass per plant is calculated using the total dry mass of shoots and the number of plants in each group (e.g. Fig. 6c).

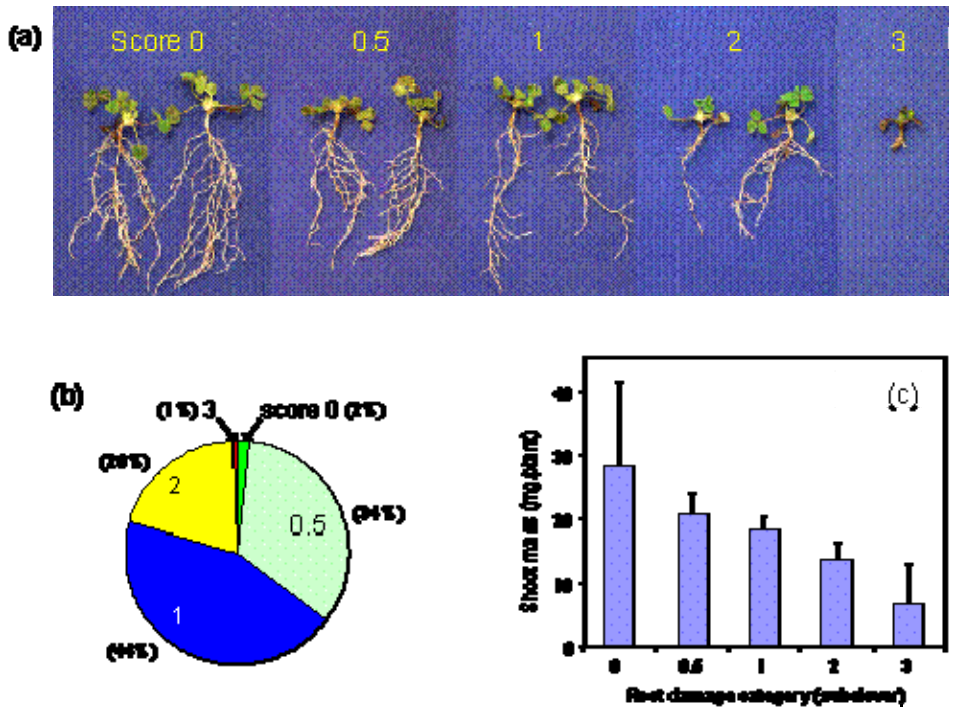


Figure 6. (a) Typical examples of subterranean clover test plants from a paddock near Hall, ACT (autumn-winter 2007), (b) proportion of surviving plants in each root damage category and (c) mass per shoot of plants in each root damage category.

$$\text{Yield achieved as a proportion of potential yield} = \frac{\text{Sum (number of plants x yield/plant in each root score category)}}{\text{Total number of surviving plants x shoot yield/plant (for score-0)}}$$

Sample calculation – From Figure 6, over the eight replicate rows there was an average of 1.3 plants/row with score-0 roots (average shoot yield 28.1 mg/plant), 25.3 with score-0.5 roots (shoot yield: 20.5), 35.5 with score-1 roots (shoot yield: 18.1), 15.9 with score -2 roots (shoot yield: 13.7) and 0.4 plants with score-3 roots (shoot yield: 6.7).

$$\text{Yield achieved as a proportion of potential yield} = \frac{(1.3*28.1) + (25.3*20.5) + (35.5*18.1) + (15.9*13.7) + (0.4*6.7)}{(78.1*28.1)}$$

$$= 0.64$$