final report

Project code:	B.PSP.0007
Prepared by:	J Shovelton MS&A K Ophell-Keller SARDI G Thomas TPS R Ballard SARDI M Ryder Ryder Research M Barbetti UWA/DAFWA R Jones UWA/DAFWA
Date published:	March 2012

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Research and Development Priorities for Southern Australian Pastures and Lucerne

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Priorities for research and development into the health of southern Australian pastures were identified from reviews of the scientific literature and from a survey of experienced agronomists. Reseach areas considered were pests, diseases and rhizobial function. Six high priority areas were selected from a wider range of issues identified. The need to maintain key research functions and improving the skills and knowledge of agronomists and producers is also highlighted.

Executive Summary

The Feedbase Investment Plan commissioned by MLA in 2011 failed to identify suitable research or development topics for investment in pasture health – specifically the adverse impact of pathogens, invertebrate pests and poor rhizobial function on the performance of pastures.

Consequently, MLA has instigated a specific review of the opportunities for investment in pasture health that will deliver tangible benefits to red meat producers. The scope of the project focusses on improved pastures in the medium-high rainfall zone and those in the mixed cropping livestock systems. Abiotic factors affecting plant health such as salinity, acidity and nutrition were not considered as part of this project except where they directly affected disease, pest or rhizobial function.

All major introduced pasture legumes as well as introduced pasture grasses were considered in the project. The impact of pathogen and pests were considered in establishing and established swards.

This project used two approaches.

Firstly, literature reviews examined the impact of biotic factors on the health, consequent productivity and persistence of the major pasture species grown in southern Australia. These reviews dealt with fungal pathogens, viral pathogens, invertebrate pests and rhizobial effectiveness

Secondly, a survey of experienced, practicing agronomists was conducted to determine their current understanding of plant health issues and their perspectives of both pasture health in general and significance of specific diseases/pests for their client base and region.

In identifying recommendations for further research and development, the views and feedback from the agronomists was considered along with the understanding of scientists of plant health issues This approach also allowed the incorporation of practical implications and management and an assessment of the capacity to do the R&D required.

The results of the survey of agronomists survey highlighted that there was a lack of knowledge by agronomists of pasture pests except for the most commonly occurring ones. The ability to recognise soil borne pests was poor. There was also generally a poor ability to quantify the impact of pests on pasture biomass or in economic terms.

The understanding of diseases was even poorer, again in terms of capacity to identify the diseases or the impacts. This applied in particular to root diseases and viral pathogens.

With regard to rhizobia, there was an assumption by agronomists that if seed had been inoculated and/or the plant had pink nodules that rhizobial function was effective. This is contrary to the scientific evidence and shows that the level of understanding by field agronomist is generally inadequate.

Scientific reviews highlighted the potential losses associated with pest diseases and ineffective/poor nodulation but there was limited data on the impact of these factors in real life field situations.

The combined effect of this lack of understanding was uncertainty as to the production and economic losses caused by these factors.

Sixteen priority areas were identifies from the agronomist survey and the scientific reviews. These investment areas were reviewed at a workshop consisting of consultants, research scientists and producers. From the workshop feedback six topics of high priority were identified.

Contents

List of figures

1. Research

The following areas require were identifies as major research priorities.

Rhizobial Function

- Quantify N limitations resulting from suboptimal rhizobial function due to across the different agro ecological zones and farming systems.
- Development of symbiotically promiscuous sub-clover genotypes
- Identification of the impact of changes in farm practices, especially herbicide impacts on fixation

Pests

- Development and implementation of IPM for Redlegged Earth Mite and blue green aphid as a means of managing the development of insecticide resistance in these pests

Diseases

- Identify strain distribution of major fungal and viral diseases and define relative resistances and/or tolerances of current and near-release breeding genotypes across all major pasture species to fungal, and viral pathogens.
- The evaluation of non-breeding approaches to fungal and viral disease management, including cultural practices.

Lower Priority Research Areas

Other areas which were considered to be of importance but of lower priority were:

- Control of white fringed weevil, particularly in lucerne. (restricted but increasing in importance)
- Control of red-headed/yellow cockchafers (focus of Dairy Australia project)
- Control of powdery mildew (localised issue)
- Breeding for resistance to new biotype of blue green aphid (subject of current screening project)
- Understand the causes of rhizobial seed coating failure and development of remedial actions. (research being undertaken by NSW DPI)

2. Capacity Building

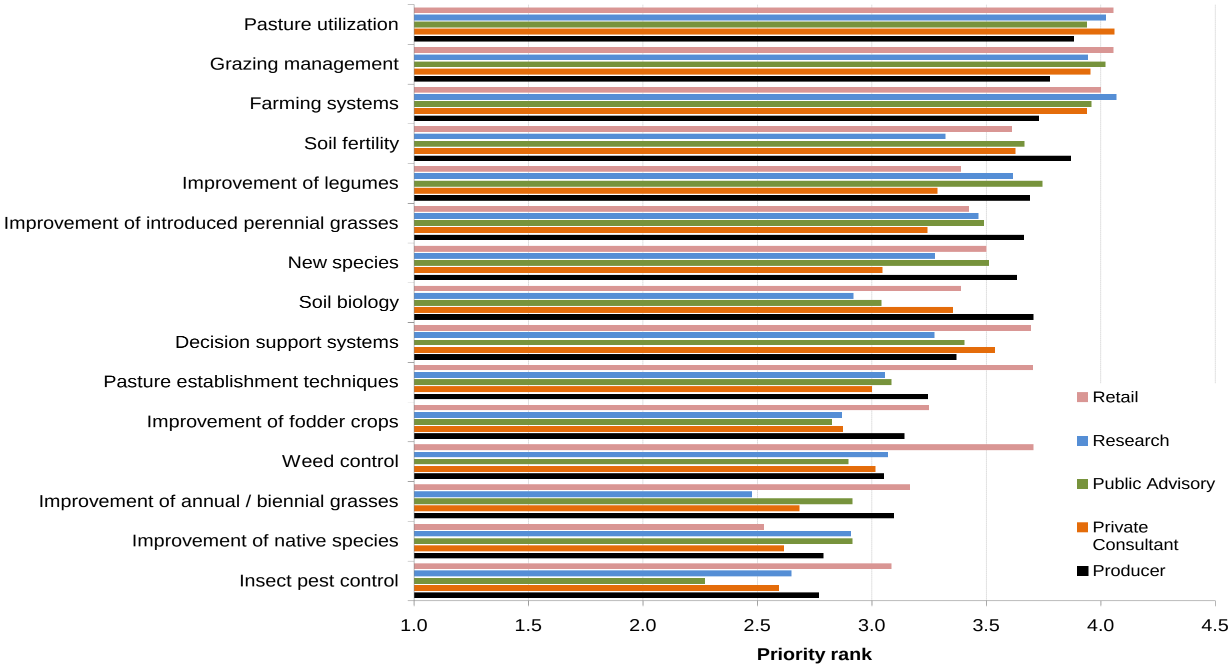
The project has highlighted serious issues in relation to both current and future research capacity in pasture health and in the capacity of agronomists to effectively identify, and therefore by implication, effectively manage pest and disease issues.

The funding of research in the areas outlined above would help address the critical level of skilled pasture pathologists and entomologists, etc. At the same time, the development of greater skills of agronomists and consultants in pasture health is recommended as a vital link in the identification and management of pasture pests and diseases and providing conduit from the field to researchers and vice versa. A joint approach is strongly recommended. It is also a critical component of educating farmers and providing them with informed advice

3. Introduction

In the 2011 Feedbase Investment Plan for southern Australia commissioned by MLA, pasture pests and diseases rated poorly as issues of importance. Over 440 farmers, consultants, researchers and extension offices responded to an on line survey which canvassed the importance of a range of investment areas for feedbase research. Figure 1 shows the low ranking given to insect pest control by the various agricultural sectors.

Figure 1: Priority components of the feedbase requiring research to increase red meat production as nominated by each sector (FIP MLA, 2011)



As well, the call for suitable pasture health projects in the FIP delivered no projects that were suitable for immediate investment. As a result, MLA has commissioned a review of published and unpublished literature and the less formal views of pasture health experts, pasture advisors and leading producers of all aspects of pasture health. The focus is on pastures used for red meat production in southern Australia, with a view to providing a sound basis for future research investments.

“Pasture health” in the context of this project has been taken to include pathogens and invertebrate pests of legume (including lucerne) and grass pastures. It has also included issues associated with effective nitrogen fixation by rhizobia.

The key assumptions underpinning the development of a pasture health theme in the Feedbase R&D Plan include the following assumptions:

- Sub-optimal pasture health is a significant cause of poor pasture performance and reduced pasture persistence.
- Pasture health issues have not been adequately quantified, and the costs to the red meat industry have not been determined.
- Pasture health is directly linked to pasture persistence issues and any developments in this theme should be implemented in association with that program of work.

In developing a series of research priorities, the views of experienced consultants across southern Australia have been combined with the scientific literature to provide a firm basis for the priorities proposed. The recommendations have been reviewed by an industry workshop prior to the development of final recommendations (to be done).

4. Method

The identification of research and development priorities for pasture health was approached from two perspectives. Firstly literature reviews were undertaken by the research establishment to identify the pest and disease issues of pastures and lucerne as well as issues associated with rhizobial efficiency. Parallel to these reviews, a phone survey was conducted with a number of agronomists in each of the agro-ecological zones to gain their perspectives pest, disease and rhizobial issues.

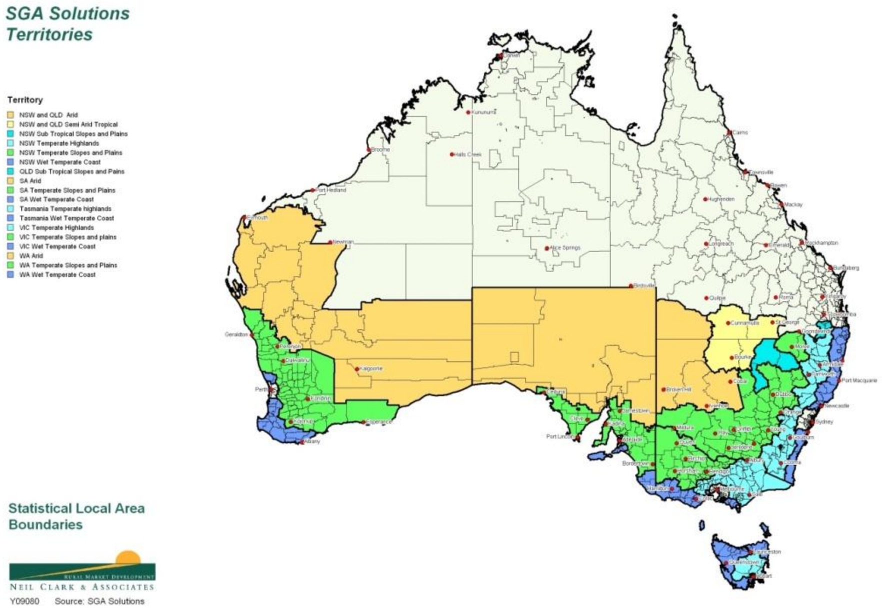
This was done to:

- Ensure that issues seen as important in the field were addressed by the research reviewers and
- To identify if there were issues identified in the reviews that were unknown or under-recognised by agronomists.

4.1 Geographical Zones Considered

The recommendations and data collection for this project were considered with regard to agro-ecological zones for southern Australia, shown in Figure 2.

Figure 2 : Agro-ecological zones



Source: SGA Solutions

Key

🟦	Wet Temperate Coast	🟦	Temperate Highlands	🟩	Temperate Slopes and Plains
🟦	Sub-tropical Slopes and Plains	🟨	Semi-arid Sub-tropical Plains	🟧	Pastoral

Figure 3 and Figure 4 respectively show the distribution of beef cattle and sheep in Australia. It can be seen that the majority of stock in southern Australia is confined to the Temperate Slopes and Plains. Consequently, the Pastoral zone and Semi-arid, Sub-tropical Plains were not considered in this report because of the relatively low numbers of stock and the lack of sown introduced pasture species.

In addition, the issues fell into two distinct zones – those where permanent pasture was most common, and the mixed farming zone where annual pastures prevailed. Lucerne was common to both.

Figure 3 : Estimated population distribution of sheep in Australia (million head) (2008). Sourced from National Sheepmeat Production RD&E Strategy, Jan 2010, PISC

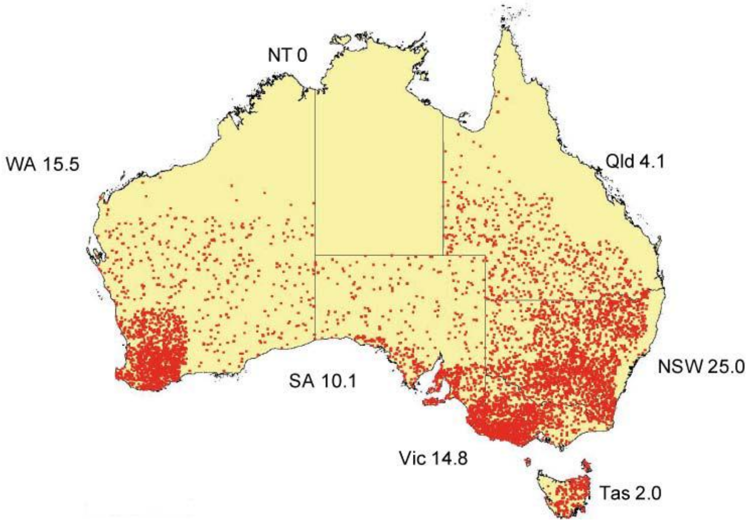
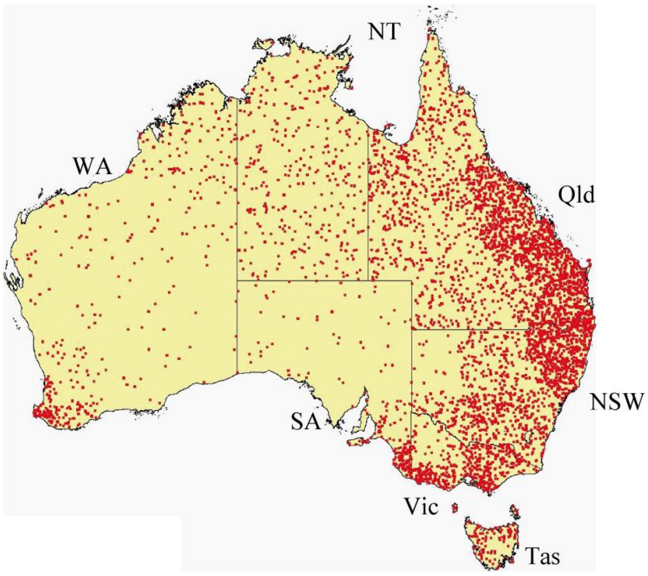


Figure 4 : Estimated population distribution of beef cattle in Australia (million head) (2008). Sourced from National Beef Cattle Production RD&E Strategy, Jan 2010, PISC



4.2 Pasture Types Considered

Rain-fed pastures only were considered. The pests and disease impacts were considered at establishment and in established pastures and lucerne, and, because of the significance of legumes in rain-fed pastures, there was a particular focus on the impact of the legume component. However consideration was also given to the introduced, perennial grass component of pastures.

As well as the results of the survey of agronomists (Appendix 2), the proposals for R&D are presented at three levels – a summary of each (Section 3 below), a more detailed “one pager” for each (Appendix 1), and the scientific reviews which provide the basis for each proposal (Appendices 3-5).

4.3 Project Outputs

The results of the survey of agronomist are provided in Appendix 2. Detailed reviews of issues associated with rhizobial effectiveness, pasture pests and pasture diseases are found in Appendices 3, 4 and 5 respectively.

4.4 Project Recommendations

A number of issues requiring further investigation were identified by combining the results of the agronomist survey with the reviews of research in pasture disease, pest and rhizobia.

These were distilled into sixteen one page descriptions (Appendix 1) of which were put to an industry workshop for consideration.

As a result the list has been further refined into 6 projects, which can be grouped in 3 areas of proposed investment:

- Defining the extent of pasture health constraints and practices
- Management of plant health by plant selection
- Improvement of plant health through management

Major topics were identified for each of the discipline areas. As well there were a number of other topics of lesser importance which are mentioned for the sake of completeness.

The paucity of economic field data on the impact of pests, diseases and rhizobia highlights a significant gap in the management of pasture pests and diseases. An essential part of any research should be greater research into the economics of losses in pastures due to pests and diseases and the economics and risks of various control strategies. This requires economic/risk management research alongside future technical projects and requires having a contracted practical economist working with researchers and consultants/agronomists to contribute to various projects from commencement and assess the various options which emerge.

4.4.1 Defining the extent of pasture health constraints:

Quantifying the extent of N limitations to legume production in different agricultural regions – Permanent Pasture Zone

Understanding the true position of N limitations in various field environments is basic to future research and extension. There is a need to broaden the field based evidence to improve awareness of N limitations to legume growth. Presently, these limitations are simply not recognised because they go unnoticed on all but the most N deficient of soils. In particular, the level of symbiotic capacity required to optimise legume growth needs to be quantified for different field environments.

Increased awareness of symbiotic limitations and opportunities to improve pasture production and nitrogen output will influence producer decisions. For example, in permanent pasture areas the circumstances in which inoculation is beneficial will be better understood and pasture legume choices refined.

The work would best be undertaken in collaboration with existing legume evaluation programs and it is suggested that a sub-set of treatments (+N or high rates of inoculation) could “value add” to the NVT trials being proposed by MLA. The potential to improve symbioses in established pastures using annual applications of inoculant should also be considered. Where substantial production responses are shown more detailed investigations of symbiotic changes (nodulation, rhizobia counts, 15N measures of fixation) would need be undertaken.

In isolation, this project would require a half time Research Officer (0.5 FTE) to assist with inoculations, sample sites and run greenhouse bio- assays. However, many of the tasks are complementary to work foreshadowed in the project focussing on changing farming systems (see above) and could therefore be delivered in concert with that project with the provision of a modest budget for casual labour and operating expenses.

Quantifying the extent of N limitations to legume production in different agricultural regions – Cropping/Pasture Zone

With the increase in cost of N fertiliser and the likely impact of carbon/energy taxes, and the increased interest in livestock on mixed farms, there is a renewed interest in the use of legume pastures in the rotation as a source of N for the system

Evolving farming systems in the cereal-livestock zone are characterised by increased herbicide use, longer cropping phases and increased application of fertiliser N. The extent to which these practices reduce nodulation, reduce nitrogen fixation, or affect other aspects of pasture legume root health need to be clarified. The impact of herbicide use is of particular concern.

Better awareness of the factors most detrimental to nodulation and N_2 fixation will provide the opportunity to make adjustments to farm practices. In the longer term the information will be critical to the development of legume cultivars that may require specific herbicide tolerances or improved tolerance of soil nitrate to perform adequately in contemporary farming systems.

Assessment should be made of basic symbiotic parameters (rhizobia number, nodulation and N_2-fixation) for farms in the cereal-livestock zone that differ in cropping intensity, herbicide and N application. Consultant networks and farming systems groups should be utilised to identify contrasting farms and poor legume performance. Existing crop sequencing trials (GRDC) could be used to indicate more specific effects of rotational sequences on pasture N_2 fixation. This would lead to focussed trials examining the most important factors in detail and the development of management guidelines.

A Research Officer would be required to co-ordinate survey activities, complete laboratory and greenhouse bio-assays, interpret results and initiate detailed studies.

Co investment should be sought from GRDC given the relevance of this work to the N economy of cereal and oil-seed crops on mixed farms.

The work in both these areas would assist in building capacity of collaborating consultants/producers to improve awareness and training in recognition of N limitations and N fixation and provide the basis for future investments.

4.4.2 Management of plant health with plant selection:

Dealing with ineffective soil rhizobia using a plant selection approach.

After water, nitrogen is the most limiting input to pasture growth. Although an abundant supply of cheap and sustainable nitrogen can be supplied by efficient nitrogen fixing symbioses, often this source of nitrogen is limited by poor compatibility between the legume host and the naturalised rhizobia in soils. This directly reduces growth of the legume as well as total pasture productivity.

There is substantial opportunity to improve the amount of nitrogen produced by sub clover symbioses, because the naturalised rhizobia in soils where clovers are grown average about 50% the fixation capacity of effective inoculant strains. This has been shown repeatedly in greenhouse trials where the masking effects of soil N have been removed to reveal some very poor symbioses.

Because the naturalised soil rhizobia are resistant to displacement by effective inoculant strains, finding sub clover genotypes that are more compatible provides the best option to improve N fixation.

Sub-clover lines should be assessed for their compatibility with naturalised soil rhizobia collected from different regions. Existing sub clover varieties should be included in this assessment to provide immediate feedback to growers. Lines showing improved compatibility with naturalised soil rhizobia should be used to demonstrate the benefits of greater N fixation to legume growth in the field and elite genotypes used to develop varieties with improved symbiotic compatibility.

Characterisation of sub-clover genotypes for their compatibility with soil rhizobia will:

- provide growers with information on the symbiotic capacity of existing varieties,
- raise awareness of symbiotic constraints to legume production,
- lead to the development of varieties with high symbiotic capacity.

A rhizobial ecologist would be required to undertake plant/rhizobia interaction studies and the selection and validation of improved sub clover lines. The work would need to be closely aligned with legume development programs and breeding support would be required to generate bred lines and ensure the combination of improved N fixation with other agronomic traits. Support would also be required for field evaluation activities in southern NSW and south-east SA/VIC.

Sub clover populations could be used for more detailed studies (e.g. PhD) aiming to understand the mechanisms controlling symbiotic compatibility.

There is potential for co-investment from the pasture seeds industry for this work.

Disease resistance of current and near-release pasture cultivars

There is a lack of disease resistance information (both for fungal and viral pathogens) for near-release breeding material. Limited disease screening is undertaken currently and many imported cultivars are screened for disease resistance in the US or Europe. This means that varieties sown and promoted in Australia are not characterised for resistance to Australian races of key pasture pathogens, and that there is no standardised disease resistance rating system.

An opportunity exists for MLA to make an investment in the development of disease screening systems which take account Australian races of key pathogens. This would involve:

- Characterisation of strains of key pasture (fungal and viral) pathogens
- Determination of distribution of strains nationally
- Development of a standardised system to screen varieties

Development of the disease screening system should be considered as part of an MLA investment in pre-breeding, and screening services could ultimately be offered to companies on a fee-for-service basis. MLA should consider the potential to link disease ratings of species with the results from the NVT program.

4.4.3 Improvement of plant health through management:

Integrated disease management

Many of the approaches to managing disease suggested by the review to are linked to incorporation of disease resistance in new varieties or management of disease at sowing. There is a need to evaluate non-breeding approaches to disease management, including cultural practices (evaluation of low cost chemical treatments, grazing, cultivation, healthy seed stocks, and manipulation of pasture composition).

There is previous research to demonstrate that these approaches can be effective:

- Apron® is registered for control of damping-off disease in some pasture legumes- however, this chemical is comparatively expensive and targets only part of the pathogen complex associated with root disease. There are a range of cheaper fungicide treatments that target a wider range of soil-borne pathogens.
- Strategic foliar application of cheap insecticides can successfully manage spread of aphid-borne viruses, including non-persistently transmitted viruses, like BYMV and AMV. This would need to be integrated into a broader IPM program.
- Research in annual legume pastures has demonstrated that soil disturbance significantly reduces root disease in sub clover for a period of three years.
- Close grazing controls rusts and powdery mildew, reducing losses by up to 25%, and reducing losses from *Kabatiella* by up to 35%; and controlled grazing can reduce losses from *Phytophthora* root rot by up to 55% in situations where this disease is particularly severe.
- Admixture with a non-host pasture species (e.g. grasses) reduces AMV spread by up to 30% in medic; and sowing of healthy seed stocks successfully manages introduction, spread and impact of seed-borne virus disorders in new medic, sub clover and lucerne pastures, especially if caused by AMV and CMV where losses are reduced by up to 35%.

There is an opportunity to evaluate a range of non-breeding disease approaches by:

- Monitoring of existing trial sites (e.g. NVT sites and existing pasture management trials)
- Field trial/demonstration sites- Potential management approaches can focus on seed quality and vigour, the selection of tolerant plants from within otherwise adapted cultivars, the application of fungicide combinations and the management of pathogen levels before sowing.
- Incorporating a demonstration sites with training opportunities with consultants and key growers.

The suggested initial focus is on soil-borne diseases-identification of root disease complexes and losses, using a combined approach of DNA assays and isolation techniques. There is a link with GRDC programs e.g. Crop Sequencing program.

There is considerable synergy with the approach identified in the potential project area with that identified in the Pasture Soil Biology Project

Development and implementation of IPM for RLEM and aphids

Pesticide resistance is an emerging problem in Redlegged Earth Mite (resistance to synthetic pyrethroids) and aphids (resistance to synthetic pyrethroids and organophosphates.

Resistance in RLEM is particular concerning given this species is the one of the most important pasture-production invertebrate pests; it attacks all grasses, clovers, medics and lucerne, reaches very high densities (e.g. in pastures > 10,000 per /m2) and is widely distributed across southern Australia (incl. Tasmania). In 2006 high levels of resistance to synthetic pyrethroids were discovered in WA. In recent study monitored resistance in field populations and found it had spread considerably within the state of WA. Twenty-six paddocks from 15 individual properties were identified with resistance, and these paddocks ranged over 480 km. The high levels of resistance occurring in WA have caused considerable economic losses due to ineffective chemical applications and mortality of seedlings at establishment. These findings highlight the need for a comprehensive resistance surveillance program to be developed. Growers need to consider non-chemical approaches for pest control and should be encouraged to implement pesticide resistance management programs.

The workshop identified the need to determine current patterns of pesticide use in pasture systems (including mixed farms) in southern Australia, to monitor the distribution and spread of existing pesticide-resistant mites (RLEM) and aphids, and finally to develop new guidelines for pesticide application that will promote the longevity of their effective use. Components that should be considered in this project area are:

- An initial survey to determine current patterns of pesticide use in pasture systems in southern Australia, including amounts of pesticide, stated target pests, timing and placement. This will involve consultants and producers.
- A monitoring program to map the distribution and rate of spread of pesticide-resistant Redlegged Earth Mite and aphids. It will also be important to find out how much of the wider incidence of resistance (Umina et al., 2012) is via spread and how much this is due to multiple, independent new occurrences of resistance. Information relating to the occurrence and spread of pesticide-resistant RLEM could also be used to develop a model to predict the future distribution and rates of spread.
- Production and dissemination of new management guidelines that incorporate strategic timing and placement of insecticides, resistant cultivars, grazing management and enhancement of natural enemies.

4.4.4 Lower Priority Research Issues

There was a number of other research issues which although important were either more restricted in their distribution or were the subject of research by other organisations. For completeness these are listed below.

Control of white fringed weevil

White fringed weevil rated very highly as a pest in the lucerne growing areas of NSW and Gippsland. There are no effective biological or insecticide control methods for this pest and it is seen to be increasing in importance.

Control of red-headed/yellow cockchafers

These pests are important in the high rainfall permanent pasture zones in eastern Australia. Red headed cockchafers are the subject of a research project funded by Dairy Australia focussing on cultural methods of control. The applicability of this approach to broad acre grazing may be limited. There still exists the potential for the use of biological control with the previously available metarhizium fungus, which is no longer commercially available due to inconsistent demand issues in the past.

Control of powdery mildew

Powdery mildew was identified as an issue of increasing importance of medics in SA. The incidence of this disease in recent years has been linked to seasonal conditions. However, the workshop did not see this as an area of national importance.

Breeding for resistance to new biotype of blue green aphid

The emergence of a new biotype of blue green aphid which has significant impact on medics as well as sub clovers is of concern. However, because there was research being undertaken at SARDI, it was decided that it would be prudent to assess the research in the short term.

Understand the causes of rhizobial seed coating failure and development of remedial actions.

The low/non-existent levels of rhizobia on seed at the point of sale were a major concern to agronomist and the research scientists. NSW DPI is currently undertaking research into the causes of failure. Opportunity exists for MLA to establish and promulgate inoculation protocols within the industry which would then be self-managed.

4.4.5 Capacity Building.

During the course of this study two things were obvious and need to be addressed as one of the essential outcomes:

Agronomists and farmers urgently need training in the identification of pests and diseases, the damage symptoms, and issues to do with rhizobial effectiveness. Without this how will they ever be able to recognise the problems they face? This requires the development of pasture pest and disease manuals and the conduct of short courses. It should be done in conjunction with some of the projects outlined above such as 'Quantification of N limitations, 'Ensuring symbiotic competence in changing farming systems and the proposed projects on 'Integrated Disease Management", and 'Extending the longevity of pesticide treatments'.

There is a general shortage of expert resources in each of the disease, pest and rhizobial research areas. This will be exacerbated by the retirement within 5 years of the senior researchers in pasture pathology in WA. One of the outcomes of this study should be to appoint suitable younger scientist at both the PhD and post-doctoral level to work with experienced researchers in order that their knowledge and experience be transferred. So that these new people understand and are encouraged in a long term commitment to the pasture industry, their training should include a strong industry and field components, including working with agronomists. Suitable projects for these linkages include 'Dealing with ineffective soil rhizobia using a plant selection approach', 'Development and implementation of IPM for RLEM and aphids' and 'Integrated disease management'.

It is recommended that:

- Training programs for agronomists be provided on a regular basis to improve their ability to identify pests and diseases and assess economic impact
- Agronomists be engaged through a research and development program of field monitoring of the presence and impacts of various pests and diseases. Such a program would have a rigorous science based protocols driven by competent research teams as its basis.
- It is recommended that farmers should work alongside agronomists in groups to play an active part in the pasture monitoring/learning process
- The approach to building research capacity needs to combine the knowledge and skills of experienced research people with field experience of consultants/agronomists. It is essential to build this relationship. Research must be based on generating real benefits for producers but at the same time transferring the skills and expertise of the experienced researchers. This is best done by funding projects at a level which allows for the salary of postdoctoral scientists and, for some projects, PhD/Masters scholarships. These projects should be closely linked to existing areas of expertise in pathology, entomology and rhizobia.
- Research Capacity – Farm Systems. Pastures have always been the core element of production systems. While the use of pastures has diminished in mixed systems in recent years with the adoption of intensive cropping, there are signs that they are returning in importance not just as a feed source for livestock but as break crops and a source of nitrogen for following crops. The integration of pastures into cropping systems and the impacts of grazing, cultivation and herbicide use need to be better understood. There is a lack of understanding of the impact of these practices in the field. Again the farmer group approach as recommended in the MLA FIP would provide the necessary feedback loops to ensure that there was rapid flow of information around the agricultural research and development network.
- Research Capacity – Farm Decisions and Economics. Little is known about farmer knowledge, attitudes and practices in pastures. One can reasonably assume from the survey data that farmer knowledge of pasture health issues is poor and that in most cases they are underperforming in pasture management, especially in pasture health. What needs to investigated is why this is so and how management decisions can be

improved. That is, we need research into farmer decision making. This would utilise the field agronomists under the guidance of a person with KASA research expertise. Again farmer groups provide a suitable basis

5. Appendix 1

Identified Research Priorities

5.1 Rhizobial Issues

5.1.1 Proposed area of investment: how much n is needed for optimal legume production?

The Issue

Quantification of the level of symbiotic capacity required to eliminate N limitations to legume growth in the field is needed to:

- Broaden the field based evidence of N limitation to legume growth.
- Raise grower and consultant awareness to symbiotic constraints.
- Direct efforts on symbiotic improvement to where it will have greatest impact.

Background

Without measurement, poor legume symbioses go unnoticed on all but the most N deficient of soils. This was apparent in responses where survey respondents initially indicated that they believed that nodulation was moderately effective to highly effective. When asked their reasons for such a statement, most admitted that they had no evidence or that they assumed because the seed was inoculated and/or that pink nodules were present, effective nodulation had taken place.

This perception is generated by inaccurate extension material and often based on rudimentary assessments of nodulation, that lead to the conclusion that pasture legume nitrogen fixation is satisfactory. This should be continuously questioned given the inconsistent relationship between nodule appearance and fixation capacity and an absence of any reliable nodulation benchmarks for comparison. While it has been recognised for a long time that sub-clover production levels fall well short of water limited potential, we are aware of only one study examining N limitations to a sub-clover pasture that was not constrained by soil acidity. It concluded that there is potential for large increases in production (9 t/ha) if N supply can be increased. There is also a body of evidence based on greenhouse studies that show the rhizobia in soils are on average 50% as effective as commercial inoculant strains, but the extent to which this is limits legume production in different field environments remains poorly understood.

Gap/opportunity

There is currently little information in the science literature that relates the symbiotic constraints attributable to ineffective rhizobia measured in greenhouse studies to the level of symbiotic needed to satisfy N demand in different field environments. N limitations are likely being underestimated in some situations and overestimated in others.

Approaches

The work should be undertaken in collaboration with existing legume evaluation programs and it is suggested that a sub-set of treatments (+N or high rates of inoculation) could “value add” to the NVT trials being proposed by MLA. Where substantial production responses are shown more detailed investigations of symbiotic constraints (nodulation, rhizobia counts, ^{15}N measures of fixation) would be undertaken.

Resources

Part time position (0.5 FTE) to assist with inoculations, sampling sites and run greenhouse bio-assays.

Risk

Low.

Timeframe

Short-term (3 years). A three year project is proposed, with a stop go clause after the first year. Response data available at the end of the first growing season used to decide project progression and direct project activities.

Outcomes

- Quantify N and symbiotic limitations to legume production.
- Establish relationships between effectiveness of soil rhizobia and N limitation to legume production in different field environments
- Increased awareness of symbiotic limitations by growers, consultants and legume development programs,.
- Added value to proposed NVT trials.

5.1.2 Proposed area of investment: dealing with ineffective soil rhizobia using a plant selection approach

The Issue

The symbiotic capacity of naturalised rhizobia in soils where clovers and medics are grown average about 50% the fixation capacity of effective inoculant strains. There are examples of very poor symbiotic associations specific to some annual legume species and regions. Soil rhizobia are likely to be responsible for more than 90% of nodulation in annual self regenerating pastures and are resistant to displacement by effective inoculant strains.

Background

Large populations of rhizobia that nodulate annual clovers and medics are widely distributed in Australian soils. Accidental introductions of rhizobia and genetic exchange in the soil has resulted in diverse but often poorly effective communities of rhizobia. For example, in the study by Drew and Ballard (2010), 32% of soils contained populations of sub-clover rhizobia that had symbiotic capacity of ≤50%, compared to the commercial inoculant strain. There is therefore potential for symbiotic improvement across several million hectares of grazing lands.

Inoculation with effective rhizobia is infrequent and generally the benefits of effective strains introduced through inoculation are temporary and possibly insignificant where the pasture phase extends past a few years.

Rhizobial effectiveness in the field was identified as a significant research issue in the survey of consultants.

Gap/opportunity

Opportunities for managing soil rhizobia are more likely to emerge from a better understanding of how different plant genotypes interact with rhizobial communities to sometimes increase nodule mass or influence which rhizobia form nodules. Various studies in both the greenhouse and field provide substantial and sometimes dramatic evidence of the potential for plants to influence symbiotic outcomes, but these possibilities have not yet been exploited at any practical level in the field in Australia.

Currently there is no information in the extension literature on the relative symbiotic capacity of different legume genotypes. Negligible information is available for many of the new clover species becoming available.

Approaches

The work should have an applied focus and be closely aligned with legume development and breeding programs to ensure that symbiotic improvement is valued and combined into agronomically fit material with commercial prospects. Selection and development of a symbiotically promiscuous sub-clover genotype would be the first priority.

Resources

A rhizobial ecologist plus support for breeding and field evaluation activities

Risk

Moderate. Background work has already been undertaken and large differences in symbiotic capacity between legume genotypes have been identified.

Timeframe

Symbiotic ratings on commercial legume varieties could be developed in 3 years. Development of symbiotically promiscuous varieties would likely take 10 years, in line with cultivar development timeframes where significant genetic gain is made.

Outcomes

- Increased legume production, stocking rate and amount of fixed N from pasture legumes forming effective symbioses with soil rhizobia.

A 10% improvement in fixed N per ha (extra 7.5 kg/ha) would enable the production of an extra 250 kg of legume DM/ha, enough to carry an addition 0.2 DSE/ha. Where pastures are used in cropping systems, the value of this extra N in fertiliser equivalents would be $10/ha.

- Availability of promiscuous sub-clover genotypes for use in symbiotic studies.
- Increased awareness of symbiotic competence by legume breeding programs.
- Increased understanding of the potential to manage soil rhizobia through plant selection.

5.1.3 Proposed area of investment: ensuring symbiotic competence in changing farming systems

The Issue

Poor nodulation is limiting contributions of fixed nitrogen and stocking rates on farms in the wheat sheep zone. What aspects of changing farming systems are affecting the establishment and functioning of pasture legume symbioses?

Background

Evolving farming systems in the wheat-sheep zone are characterised by increased herbicide use, longer cropping phases and increased application of fertiliser N. All these factors are known to impact adversely on either the establishment or function of legume symbioses. Reports of poor medic nodulation and recent confirmation of this in several evaluation trials indicate that some aspect of system change is affecting symbiotic performance. Poorly nodulated plants produced 35% less early season growth when feed availability is critical to stocking rate and has the potential to reduce the amount of N fixed by 40 kg/ha. The reasons remain to be elucidated.

Survey respondents identified survival of rhizobia in the field as influenced by either acid soil conditions, cropping rotations and the use of herbicides as an areas of concern.

Gap/opportunity

Knowledge gaps exist around the effects of changing farming systems on the symbiosis. Studies have measured number of rhizobia in the soil and found this to be satisfactory, but have not been able to reconcile this finding with observations of poor nodulation. More subtle effects on nodulation processes are likely to be involved. While herbicides are known to affect infection sites on legume roots and have been observed to disrupt the function of established nodules, there have been few systematic investigations on N_2 fixation and should be a focus of investigations of management practices that affect symbiosis.

Approaches

An assessment should be made of basic symbiotic parameters (rhizobia number, nodulation and N_2-fixation) for farms in the wheat sheep zone that differ in crop intensity, herbicide and N application. Consultant networks should be utilised to identify contrasting farms and poor legume performance. Existing crop sequencing trials (GRDC) could be used to indicate more specific effects of rotational sequences on pasture N_2 fixation. Specific studies of herbicide impact should also target pastures in the wheat sheep zone, where effects are likely to be greatest because of the diversity and amount of herbicide applied. The work should include herbicides presently considered benign (e.g. grass selective) because so far only rudimentary assessments have been undertaken. Symbiotic function of legume genotypes being developed to tolerate herbicides should be assessed.

Resources

Field work would best be incorporated into existing crop sequencing and pasture legume programs and funds would be required for sampling of existing trials and establishment and maintenance of supplementary trials. A position (1 FTE) would be required to co-ordinate activities, sample sites and complete laboratory and greenhouse bio-assays.

Risk
Low

Timeframe
5 years

Outcomes

- Improved nodulation and early season pasture vigour for better stocking capacity and weed competition.
- Increased supply of fixed nitrogen to the farming system and associated reductions in carbon footprint (5 kg CO_2 saved per kg N_2 fixed).
- Changed practices by farmers, agronomists and plant breeders in response to data demonstrating herbicide impacts on N_2 fixation.

5.1.4 Proposed area of investment: define ph thresholds for adequate symbiosis of different pasture legumes

The Issue
Continued soil acidification will reduce legume N_2 fixation in both the permanent pasture and wheat sheep zones and reduce stocking rate.

Background
Soils are projected to continue to acidify under current agricultural practices and small changes in pH can have profound impacts on N_2 fixation. Components of the symbiosis (survival of the rhizobia and processes of nodulation) are usually more sensitive to low pH than the plant itself and so N_2 fixation is often compromised before there are observable effects on plant growth.

The extent to which low pH is already impacting on N_2 fixation, variation between legume symbioses and adaptation of current inoculant strains all require clarification. This will be critical to maintaining effective legume pastures where lime is not able to be to be economically applied or sub-soils are acidic. There is large opportunity to increase the use of some legume species (lucerne) if improvements to their acidity tolerance can be made.

Gap/opportunity
A better understanding is needed of critical pH thresholds for adequate N_2 fixation by the suite of pasture legumes being grown in each livestock region.

Approaches
In collaboration with agronomists/consultants undertake a survey of N_2 fixation by medic and clover pastures at contrasting pH levels in the permanent pasture and wheat sheep zones. Follow up initial survey with measures of rhizobia and nodulation at selected sites. Use the information to direct efforts to improve acidity tolerance of the symbiosis and develop strategies that maintain pH above critical levels. Stress tests of the current inoculant strains should be undertaken to ensure they remain suitably adapted.

Resources

Collaboration with agronomists. Part time position (0.5 FTE) to assist with co-ordination of activities, sample preparation and run greenhouse bio-assays. Additional resourcing would be required for work to improve acidity tolerance, particularly if targeted at the low pH grazing lands in south-eastern Australia (see Capacity section).

Risk

Low/medium

Timeframe

5 years

Outcomes

Quantification of critical pH thresholds for key pasture species. Measures of inoculant strain adaptation and opportunity for improvement.

5.1.5 Potential area of investment – investigation of the causes of rhizobial seed coating failure

Identified gap

Agronomists interviewed indicated a high level of uncertainty and disquiet about the effectiveness of rhizobial seed coatings. It was unclear whether this was an issue of quality control somewhere along the supply chain or if the techniques used were effective or being used out of specification, or whether the new strains could not compete with naturalised strains. The effective introduction of new rhizobial strains of is of fundamental importance and underpins the successful establishment of species new to an environment.

Other issues

The continued development of new strains was encouraged and the ability to introduce new strains into existing swards or into swards where rhizobia were absent or in low levels was also canvassed by agronomists

5.2 Pasture diseases

5.2.1 Proposed area of investment: dealing with pathogen strain/race variation to ensure effective resistance deployment and thereby secure future pasture productivity

The issue

Lack of information relating to strain/race structures for the fungal pathogens (*Phytophthora, Kabatiella)* and major virus pathogens (SCMoV, BYMV, AMV) prevents development and deployment of strain specific host resistances to the prevailing strains/races of these pathogens. Until the naturally occurring strains/races for these 5 pathogens are defined, deployment of disease resistant varieties will be compromised such that losses will continue.

Background and Scale of Benefit

Losses caused by these pathogens in some pasture legumes have been well documented for decades (see Barbetti et al. 2005, Barbetti and Jones 2011, Jones reviews 1996, 2004, Barbetti et al. reviews of 2005, 2007 and MLA reports of 2005 by Barbetti et al. and 2011 by Barbetti and Jones). For example, post-emergence damping-off combined with severe root disease in sub clover pastures caused by *Phytophthora clandestina* is an over-riding biotic constraint to productive sub clover pastures across southern Australia constituting an important cause of pasture deterioration - the underlying issues are that all known resistance sources have been overcome by new races of this pathogen and that current status of races is unknown. There is an urgent need to both characterise races present and identify effective host resistance to them. All pasture legumes are susceptible to *Phytophthora*, with losses of up to 70% in sub clover alone throughout high rainfall regions (>500mm annual rainfall), and even up to 25% losses across low rainfall regions. There are about 20 m ha of sub clover pastures alone currently infested with *Phytophthora*. Similarly, for *Kabatiella*, >0.8 m ha is seriously affected annually with an additional 1.2 m ha significantly impacted in other high rainfall regions (>600mm) - losses up to 60% are common in high rainfall regions (>600mm). Another example is the need to address the losses caused by SCMoV and BYMV in subterranean clover pastures (up to 60% losses; costing $180 million annually to the Australian meat industry); and by AMV in medic, lucerne, and white clover pastures (e.g. up to 32% herbage and seed losses in medic swards). Effective host resistance is not currently deployed.

Gap/Opportunity

Define the pathogen strain/race structures for *Phytophthora, Kabatiella* and major virus pathogens (SCMoV, BYMV, AMV) across southern Australia, and associated host resistances against specific strains and races of these pathogens such that available host resistances can be strategically and effectively deployed.

Approaches

Survey pastures across southern Australia to characterise strains/races of these pathogens and their geographic distributions. Subsequently, screen genotypes of major pasture legumes as appropriate against each pathogen to identify effective and reliable resistance to the prevailing strains/races and to allow effective deployment of pasture species with strain/race-specific resistances.

Resources and linkages needed

This can be addressed and delivered through current plant pathology expertise at UWA-DAFWA in association with agronomy, breeding and cultivar development, and extension programs and personnel at DAFWA and elsewhere across southern Australia. This includes work MLA is currently considering for funding in association with programs targeting better pasture legume species, such as those in WA.

The Risk

There is a nil technical or capability failure risk due to excellent skills and facilities currently available in WA.

Time Scale

Surveys could commence in autumn 2012 and continue throughout the rest of 2012. Pathogen isolates would then be characterised in terms of their strain/race types and then genotype screening would follow and be completed in 2-3 additional years (dependent upon level of funding support). There is certainty for defining pathogen strains/races and for locating effective resistance to these strains; with an extremely high probability of finding very high levels of host resistance in pasture legume genotypes against all prevailing major pathogen strains. There is an abundance of excellent germplasm that can be accessed that already has known good agronomic adaptation, especially so in sub clover (fungal and viral) and lucerne (viral).

Outcomes and Benefits

Outcome will be delivery of pathogen resistant lines to breeders to breed resistant varieties and identification of any existing pathogen-resistant varieties for immediate deployment across high rainfall (>500mm) southern Australia – underpinning Integrated Disease Management for these diseases. To quantify benefits, once pathogen strains/races have been characterised and resistance to them identified, small plots should be set up nationally in different geographical regions for varieties with resistance. This will be one of the best investments MLA could ever make in terms of improving the volume and quality of the pasture feed-base, particularly at the critical autumn period of feed shortage. The expected improvement in the pasture feed-base for meat producers across high rainfall regions of southern Australia will be in the order of 35% or above.

5.2.2 Proposed area of investment: defining resistances to major diseases in near-release breeding genotypes of all major pasture species to secure future pasture productivity

The Issue

Lack of information relating to relative resistances and/or tolerances of all near-release breeding genotypes across all pasture species to significant fungal pathogens other than Phytophthora and Kabatiella as covered in investment summary #1 (viz. Pythium, Cercospora, rust, powdery mildew, Aphanomyces), and viral (SCRLV, BYDV, WCMV, RyMV) pathogens prevents effective development and deployment of host resistances. Until better sources of resistance to these pathogens are identified for breeders to utilise, severe losses will continue.

Background and Scale of Benefit

Losses from inadequate host resistance to diseases have been well documented for sub clover and annual medics for decades (see Barbetti et al. MLA report 2005; Barbetti and Jones 2011; Barbetti et al. reviews 2005, 2007; Jones reviews 1996, 2004). For example, pre- and post-emergence damping-off by Pythium, and damping-off and root disease from Phytophthora, result in pastures failing to establish or persist, or be productive in high rainfall (>500mm) regions across southern Australia, with annual average losses up to 40-45% losses in the most disease prone regions. Losses caused by Cercospora (up to 20% loss in sub clover in high and medium rainfall regions), rust (up to 20% loss in high and 10% loss in medium rainfall regions), powdery mildew (10% loss in some south coastal regions of southern Australia) and Aphanomyces (up to 35% losses in high rainfall regions >600mm). Another example is the major losses from virus diseases in simulated swards or spaced plant trials, particularly - BYDV and RyMV in perennial grasses (e.g. ryegrass reduced by up to 50% in swards by RyMV infection), WCMV in white clover (24% herbage, 71% root nodule losses) and SCRLV in sub clover (up to 61% herbage loss). Conservatively, the combined annual losses from major fungal and viral diseases of legume based pastures across southern Australia are likely to exceed $800K annually.

Gap/Opportunity

For major fungal and viral pasture diseases, define relative resistances and/or tolerances of all near-release breeding genotypes across all major pasture species to other major fungal (Pythium, Cercospora, rust, powdery mildew, Aphanomyces), and viral (SCRLV, BYDV, WCMV, RyMV) pathogens, and identify sources of resistance to these pathogens for breeders to utilise.

Approaches

Screen all near-release breeding genotypes across all major pasture species to other major fungal (Pythium, Cercospora, rust, powdery mildew, Aphanomyces), and viral (SCRLV, BYDV, WCMV, RyMV) pathogens, and identify sources of resistance to these pathogens for breeders to utilise in breeding new varieties.

Resources and linkages needed

This can be addressed and delivered through current pathology expertise at UWA-DAFWA in association with agronomy, breeding and cultivar development programs across Australia, including work MLA is currently considering for funding in association with national programs targeting better pasture legume species. A combination of controlled environment and field screenings will be undertaken.

The Risk

There is a nil risk of failure from lack of skills or experience or suitable facilities due to excellent skills and facilities currently available in WA.

Time Scale

Genotype screening against all these nine pathogens could be completed in 3 years (dependent upon level of funding support). There is high probability for locating effective resistance against all these pathogens (Barbetti and Jones, unpublished) and an extremely high probability of locating very high levels of host resistance in diverse pasture legume and grass genotypes. There is an abundance of excellent germplasm that can be accessed that already has known good agronomic adaptation.

Outcomes and Benefits

Outcome will be deployment across southern Australia of effective host resistance to these additional major nine pathogens – underpinning Integrated Disease Management for these diseases. To measure this, once host resistance to them has been identified, small plots will be set up nationally for demonstrating varieties with resistance in different geographical regions to quantify value of resistance and investment – this will be a very high return investment for MLA, expected to conservatively improve the productivity of the pasture feed-base for meat producers across high rainfall regions of southern Australia in the order of 35% or more.

5.2.3 Proposed area of investment: defining resistances to major diseases in near-release breeding genotypes of all major pasture species to secure future pasture productivity

The Issue

Lack of information relating to relative resistances and/or tolerances of all near-release breeding genotypes across all pasture species to significant fungal pathogens other than *Phytophthora* and *Kabatiella* as covered in investment summary #1 (viz. *Pythium, Cercospora*, rust, powdery mildew, *Aphanomyces*), and viral (SCRLV, BYDV, WCMV, RyMV) pathogens prevents effective development and deployment of host resistances. Until better sources of resistance to these pathogens are identified for breeders to utilise, severe losses will continue.

Background and Scale of Benefit

Losses from inadequate host resistance to diseases have been well documented for sub clover and annual medics for decades (see Barbetti et al. MLA report 2005; Barbetti and Jones 2011; Barbetti et al. reviews 2005, 2007; Jones reviews 1996, 2004). For example, pre- and post-emergence damping-off by *Pythium*, and damping-off and root disease from *Phytophthora*, result in pastures failing to establish or persist, or be productive in high rainfall (>500mm) regions across southern Australia, with annual average losses up to 40-45% losses in the most disease prone regions. Losses caused by Cercospora (up to 20% loss in sub clover in high and medium rainfall regions), rust (up to 20% loss in high and 10% loss in medium rainfall regions), powdery mildew (10% loss in some south coastal regions of southern Australia) and *Aphanomyces* (up to 35% losses in high rainfall regions >600mm). Another example is the major losses from virus diseases in simulated swards or spaced plant trials, particularly - BYDV and RyMV in perennial grasses (e.g. ryegrass reduced by up to 50% in swards by RyMV infection), WCMV in white clover (24% herbage, 71% root nodule losses) and SCRLV in sub clover (up to 61% herbage loss). The combined annual losses from major fungal and viral diseases of legume based pastures across southern Australia could be up to $800 million annually.

Gap/Opportunity

For major fungal and viral pasture diseases, define relative resistances and/or tolerances of all near-release breeding genotypes across all major pasture species to other major fungal (*Pythium, Cercospora*, rust, powdery mildew, *Aphanomyces*), and viral (SCRLV, BYDV, WCMV, RyMV) pathogens, and identify sources of resistance to these pathogens for breeders to utilise.

Approaches

Screen all near-release breeding genotypes across all major pasture species to other major fungal (*Pythium, Cercospora*, rust, powdery mildew, *Aphanomyces*), and viral (SCRLV, BYDV, WCMV, RyMV) pathogens, and identify sources of resistance to these pathogens for breeders to utilise in breeding new varieties.

Resources and linkages needed

This can be addressed and delivered through current pathology expertise at UWA-DAFWA in association with agronomy, breeding and cultivar development programs across Australia, including work MLA is currently considering for funding in association with national programs targeting better pasture legume species. A combination of controlled environment and field screenings will be undertaken.

The Risk

There is a nil risk of failure from lack of skills or experience or suitable facilities due to excellent skills and facilities currently available in WA.

Time Scale

Genotype screening against all these nine pathogens could be completed in 3 years (dependent upon level of funding support). There is high probability for locating effective resistance against all these pathogens (Barbetti and Jones, unpublished) and an extremely high probability of locating very high levels of host resistance in diverse pasture legume and grass genotypes. There is an abundance of excellent germplasm that can be accessed that already has known good agronomic adaptation.

Outcomes and Benefits

Outcome will be deployment across southern Australia of effective host resistance to these additional major nine pathogens – underpinning Integrated Disease Management for these diseases. To measure this, once host resistance to them has been identified, small plots will be set up nationally for demonstrating varieties with resistance in different geographical regions to quantify value of resistance and investment – this will be a very high return investment for MLA, expected to conservatively improve the productivity of the pasture feed-base for meat producers across high rainfall regions of southern Australia in the order of 35% or more.

5.2.4 Proposed area of investment: defining incidence and economic importance caused by major fungal and viral diseases in lucerne, white clover, alternative annual pasture legumes and perennial pasture grasses

The Issue

There is a lack of sufficient information relating to incidence and impacts (losses and economic importance) of major fungal and viral diseases in lucerne, white clover, alternative annual pasture legumes and perennial pasture grasses across southern Australia. This information is fundamental to defining priorities for these pasture legume types.

Background and Scale of Benefit

While fungal and viral disease-induced losses have been documented for decades for sub clover and to a lesser extent for annual medic pastures (e.g., soilborne disease impacts largely undocumented for annual medic) (see Barbetti et al. 2005 and Barbetti and Jones 2011 MLA reports; Barbetti et al. reviews of 2005, 2007; Jones reviews of 2000 and 2004a), the situation regarding lucerne, white clover, alternative annual pasture legumes and grasses is unclear. For example with fungal diseases in pastures, lucerne, white clover and alternative annual legumes are very susceptible to pre- and post-emergence damping-off by *Pythium*, and root disease caused by *Phytophthora*. These pastures frequently fail to persist or be productive, particularly in high rainfall regions >500mm in situations where annual losses up to 40-50% occur for sub clover. Other examples are losses caused by AMV in lucerne, and WCMV and AMV in white clover swards. From spaced plant studies, these are estimated at 20-40%. From simulated sward studies, losses caused by BYDV and RyMV in perennial ryegrass are up to 50%. In lucerne, white cover and perennial pasture grasses, a clearer picture of virus-induced losses is needed which can only come from sown swards defoliated by grazing or mowing. No information is available as yet on losses caused by viruses in alternative annual legume pastures.

Gap/Opportunity

Determine incidence and impacts (losses and economic importance) of major fungal and viral diseases in lucerne, white clover, alternative annual pasture legumes and perennial pasture grasses (and also annual medic for soilborne diseases).

Approaches

Nationally survey for incidence and establish field trials to assess impacts (losses and economic importance) of major fungal and viral diseases in lucerne, white clover, alternative annual pasture legumes and perennial pasture grasses (and also annual medic for soilborne diseases) across southern Australia.

Resources and linkages needed:

This can be addressed and delivered through current plant pathology expertise at UWA-DAFWA in association with other relevant state and CSIRO pathology groups, pasture agronomy, pasture breeding and pasture cultivar development programs across Australia. A combination of national surveys, controlled environment and field trial studies with grazed swards will be undertaken.

The Risk

There is a nil risk of failure from lack of skills, experience or suitable facilities as these are readily available.

Time Scale

National surveys of incidence and field damage could commence in autumn 2012 and continue throughout the rest of 2012. Yield loss assessments could be completed in 3 years (dependent upon level of funding support). There is certainty of defining incidence and impact of the major diseases occurring in lucerne, white clover, alternative annual pasture legumes and perennial pasture grasses (and also annual medic for soilborne diseases) across southern Australia because the researchers have strong track records in this regard. Appropriate computer models will provide the dollar losses based on disease incidences and yield loss data from grazed swards, as used previously for virus disease losses in subterranean clover pastures (Jones 1996).

Outcomes and Benefits

Outcome for meat producers will be quantification of the importance and economic impact of major fungal and viral diseases in lucerne, white clover, alternative annual pasture legumes and perennial pasture grasses (and also annual medic for soilborne diseases), identifying key targets for future funding decisions. For fungal diseases, it is already established that in disease-favourable high rainfall areas for sub clover up to 30-90% of seedlings fail to emerge, 10-40% of surviving seedlings succumb post-emergence, with a 35-70% reduction in growth of survivors. Field observations suggest the same is happening for lucerne, white clover, alternative annual pasture legumes and for some pasture grasses. Similarly, all seed stocks of lucerne sown are contaminated with AMV, white clover pastures have high incidences of AMV and WCMV, and perennial ryegrasses have high levels of RyMV infection. BYMV is common in alternative annual pasture legume pastures. Failure of pasture species to establish successfully and the damage caused by virus diseases are a prime constraint to establishment of a productive and high quality feedbase involving one or more of lucerne, white clover, alternative annual legumes and pasture grasses (and also annual medic for soilborne diseases) for meat producers, and the incidence and impacts of these constraints need to be quantified so that future research priorities for these species can be defined.

5.2.5 Proposed area of investment: identify, evaluate and demonstrate benefits of cultural control measures for disease management

The Issue

There are many potential yet rarely exploited opportunities for utilizing manipulation of cultural practices for disease management in pasture legumes (see MLA reports of Barbetti et al. 2005; Barbetti and Jones 2011; Barbetti et al. reviews of 2005, 2007; Jones reviews 1996 and 2004). While the low level of utilisation of cultural management measures by meat producers arises partly from lack of awareness of their benefits, this is also partly a consequence of additional measures not having been identified or evaluated for their potential benefits.

Background and Scale of Benefit

Some rarely exploited opportunities for utilizing manipulation of cultural practices for management of fungal and viral diseases in legume pasture were reported previously (see MLA reports Barbetti et al. 2005 and Barbetti and Jones 2011; Barbetti et al. reviews of 2005, 2007; Jones review 2004). For example, with annual legume pastures, soil disturbance significantly reduces levels of damping-off and root disease in sub clover for a period of three years; close grazing controls rusts and powdery mildew, reducing losses by up to 25%, and reducing losses from *Kabatiella* by up to 35%; and controlled grazing can reduce losses from *Phytophthora* root rot by up to 55% in situations where this disease is particularly severe. Also admixture with a non-host pasture species (e.g. grasses) reduces AMV spread by up to 30% in medic; and sowing of healthy seed stocks successfully manages introduction, spread and impact of seed-borne virus disorders in new medic, sub clover and lucerne pastures, especially if caused by AMV and CMV where losses are reduced by up to 35%. In addition, virus infection of the legume component within pastures results in preponderance of weeds, e.g. AMV infection caused the balance of capeweed to medic to change to 90% capeweed.

Gap/Opportunity

Identify and demonstrate the benefits to meat producers from modified cultural practices (e.g., grazing, cultivation, healthy seed stocks, and manipulation of pasture composition) for reducing severity and impact of major fungal and viral diseases in pastures across southern Australia. Establish best practices for ensuring that seed stocks of new cultivar releases of all pasture legumes are free of seed-borne virus infection.

Approaches

First, identify the best prospects for cultural control measures (e.g., grazing, cultivation, altered pasture composition, healthy seed stocks), and test their effectiveness in small plots in field situations. The benefits from altering cultural practices would then be demonstrated to meat producers in reducing severity and impact of major fungal and viral diseases in pastures.

Resources and linkages needed

This can be addressed and delivered through current plant pathology expertise at UWA-DAFWA in association with pasture agronomy and extension programs across southern Australia.

The Risk

There is a nil risk of failure from lack of skills, experience or suitable facilities as these are all readily available.

Time Scale

The best prospects for cultural control measures (e.g., grazing, cultivation, healthy seed stocks, altered pasture composition) would be quickly and easily identified from the literature (Australia and overseas) and the local researcher knowledge base; then their effectiveness would be evaluated in small plots in field situations over 3 years (in particular such that impacts on soil-borne and viral diseases would be fully assessed over time and such that benefits from combining cultural with other control measures would also be evaluated), and, finally, demonstrate the benefits of the most successful measures to meat producers in larger on-farm trials run nationally in the final year. There is great likelihood for success with locating and defining successful cultural practices against the major diseases occurring across southern Australia because the researchers involved already have strong track records in this area.

Outcomes and Benefits

The outcomes will be a range of effective and minimal cost management options for meat producers to minimise the impact of root and foliar diseases on pasture legume productivity across all regions, particularly high rainfall >500mm ones. Further, this will provide meat producers with greater flexibility and choice in terms of management options and approaches available to them. Widespread utilisation of cultural control options could be expected to improve the productivity of the pasture feed-base for meat producers across high rainfall regions of southern Australia in the order of 20%.

5.2.6 Proposed area of investment: identify, evaluate and demonstrate benefits of cost-effective chemical treatments for control of fungal damping-off and viral pathogen vectors

The Issue

There exist significant but rarely exploited opportunities for utilizing low cost chemical seed treatments or foliar chemical applications to not only ensure successful stand establishment when sowing pastures (see MLA reports of Barbetti et al. 2005 and Barbetti and Jones 2011; Barbetti et al. reviews of 2005, 2007; Jones 1996 and 2004), but also for control of virus vectors. While Apron® is registered for control of damping-off disease in some pasture legumes, this chemical is comparatively expensive and targets only part of the pathogen complex associated with damping-off. There are many alternative fungicide treatments that not only would be more effective as they target a much wider range of soil-borne pathogens associated with damping-off, but also would be much cheaper, encouraging wide usage. Low cost pyrethroid insecticide treatments have proved very effective in managing aphid-borne AMV in medic pastures. The current poor level of utilisation of cheap and available chemicals for both soil-borne and foliar disease management by farmers is due to lack of knowledge of their likely benefits. There is also the challenge that several cheap and potentially more effective possibilities have not been identified or evaluated for potential roles in fungal or viral disease management.

Background and Scale of Benefit

Potential for use of chemicals for management of fungal and aphid-vectored viral diseases in legume or grass pastures was described in MLA reports of Barbetti et al. (2005) and Barbetti and Jones (2011), the Barbetti et al. reviews of 2005, 2007; Jones 1996 and 2004a). In particular, with annual pasture legumes, there are simple and cheap fungicidal seed treatments experimentally evaluated that could ensure successful stand establishment of pasture legumes, providing additional benefits reducing levels of root disease in established plants. Benefits would be extensive particularly in the high rainfall regions (>500mm), where pre- and post-emergence damping-off by *Pythium*, and root disease from *Phytophthora*, currently result in pastures sometimes failing to persist or be productive and where annual losses of up to 40-45% losses occur in the most disease prone areas. Strategic foliar application of cheap insecticides can successfully manage spread of aphid-borne viruses, including non-persistently transmitted viruses, like BYMV and AMV, and prevent them from causing losses in herbage and seed yields of up to 30% (AMV in medic) and 60% (BYMV in sub clover). Benefits will be significant as losses caused by aphid-borne AMV in lucerne, white clover and medic swards, and BYDV in perennial ryegrass swards are currently estimated at 30% or more, and losses from BYMV in sub clover swards are up to 60%. However, contact (WCMV, SCMoV) or mite transmitted (RyMV) viruses are not controlled by insecticide application.

Gap/Opportunity

Demonstrate cost-effective (i), chemical seed treatments for effective control of pre- and post-emergence damping-off of pasture legumes when re-sowing pastures and, (ii), foliar applications for controlling virus spread by aphid vectors.

Approaches
First, identify the best chemical prospects for seed treatments for control of seedling damping-off and foliar application of chemicals to control virus spread by aphid vectors; then test their effectiveness: (i) under controlled environmental conditions; (ii) in small plots in field situations, and (iii) demonstrate nationally the benefits to meat producers from these chemical treatments for ensuring successful stand establishment (damping-off and seedling root disease) and in reducing incidence and losses from major viral diseases in pastures.

Resources and linkages needed
This can be addressed and delivered through current plant pathology expertise at UWA-DAFWA in association with pasture agronomy and extension programs across southern Australia.

The Risk
There is a nil risk of failure from lack of skills, experience or suitable facilities as these are all readily available.

Time Scale
Identify the best prospects for seed treatments for control of seedling damping-off and for aphid vector control from existing literature (Australia and overseas) and the local researcher knowledge base. Evaluate their effectiveness in pots first and subsequently in small plots in field situations over 3 years. Finally, the benefits of these chemical practices for successfully establishing pasture stands and for reducing viral disease induced losses will be demonstrated to meat producers in larger trials in the final year. There is considerable certainty for success with locating and defining successful chemical treatments for these uses because the researchers already have strong track records in this area.

Outcomes and Benefits
Outcome will be a range of effective and minimal cost chemical management options for meat producers, not only to provide assurance in establishing pastures, but also in minimising root and foliar pasture diseases, particularly across high rainfall regions. Cheap and cost-effective chemical control options are particularly beneficial wherever pastures are sown/resown. These chemicals will provide meat producers with greater flexibility in terms of approaches available to them. Widespread utilisation of chemical control options across high rainfall pastures could be expected to improve the productivity of the pasture feed-base for meat producers in the order of 15%.

5.2.7 Proposed area of investment 7: retaining and rebuilding critical pathology expertise needed to support healthy pastures across southern Australia

The Issue
Barbetti and Jones are the only remaining pasture plant pathologists in southern Australia with combined competency in fungal, bacterial, viral, phytoplasmal and nematode pathogens. They have a combined 7 decades of successful IDM outcomes and package delivery for the benefit of Australian pasture-based industries across the disease spectrum of annual and perennial pasture legumes and grasses of southern Australia. This expertise will be lost once Barbetti and Jones retire in ~ 5 years' time.

Background
Maintaining the productivity and security of the pasture feed base is critical for ongoing profitability of Australia's livestock industries. Economic losses to pasture-based industries from diseases are well documented (see MLA reviews of Barbetti et al. 2005 and Barbetti and Jones 2011; Barbetti et al reviews of 2005, 2007 and Jones reviews of 1996 and 2004). Effective management of disease constraints is vital for profitability. Furthermore, there is an ongoing need for ensuring that any new pasture disease threats can be dealt with competently and expediently to minimise potential unforseen income losses.

Gap/Opportunity

There is an urgent need to train pasture pathologists and capture the existing pasture pathology knowledge of Barbetti and Jones, in order to safeguard the health of the feed-base across Australia from major disease losses into the future.

Approaches

To train/transfer, within the broader context of pasture breeding and science, broad spectrum pathology skills to: (i), a young postdoctoral fellow with almost immediate benefit to the pasture-based meat industries, and, (ii), several PhD's to ensure a wider base of expertise. Together, these new pathologists will maintain the long-term capacity of pasture pathology nationally towards ensuring productivity of the Australian livestock feed-base. Barbetti and Jones would transfer their extensive pasture pathology experience to both the postdoctoral fellow and PhD students. Barbetti is involved in undergraduate teaching of pasture systems and both Barbetti and Jones are involved with training PhD candidates on pathology issues.

Resources and linkages needed

Barbetti and Jones have comprehensive local, national and international networks with other Plant Pathologists and pasture researchers. Additional important linkages in place include (i), those with Phil Nichols (DAFWA/CLIMA) who has been a pasture legume Plant Breeder on several national plant breeding programs, including NAPLIP and the FFI CRC salinity tolerance project, and who has extensive national and international pasture breeding networks and strong links to the Australian pasture seed industry; and (ii), those with Megan Ryan (UWA) who has important national and international pasture research networks, particularly through her leadership role in the FFI CRC. These provide access to other important collaborative pasture breeding and selection programs at SARDI (including the lucerne program), DPI-Vic and NSW-DPII. Further, linkages extend to the phosphorus-efficient pasture systems project of Richard Simpson, Megan Ryan and Phil Nichols et al., providing additional opportunities to develop relevant skills.

The Risk

There is a nil technical or capability risk of failure due to excellent skills and facilities available in WA.

Time Scale

A postdoctoral trainee could be appointed immediately in 2012 upon funding being available and, if also supported by MLA, PhD candidates could quickly be placed within the different disciplines associated with pasture pathology at UWA in 2012. The minimum period required at UWA for a post-doctoral trainee is 3 years and each PhD candidate would take approximately 3.5 years to complete.

Outcome - and how measured

Having trained pasture pathologists who have captured the existing pasture pathology knowledge of Barbetti, Jones and others as outlined above, the project will provide the ongoing critical pasture pathology expertise to safeguard the health of the feed-base from major disease losses across Australia into the future.

5.2.8 Proposed area of investment: powdery mildew management

The issue

Powdery mildew (PM) is increasing in incidence and severity, causing complete pasture failure on some parts of Eyre Peninsula. It has the potential to cause serious losses of feed on offer and have longer term effects on pasture persistence on more than 5 M ha of low rainfall alkaline soils where medics, particularly *M. littoralis* is particularly susceptible. It is also a serious medic pathogen in Queensland, and potentially more broadly.

Background

Powdery mildew appears to be increasing in incidence and severity since first being reported as an emerging issue in South Australia by Howie (NAPLIP workshops, 1999). Infection can occur early in the season and has been of sufficient severity to cause stock agistment by some farmers on Eyre Peninsula. The most generally well adapted species for low rainfall alkaline sandy loam soils, M. littoralis, is highly susceptible. This issue is exacerbated by the very similar genetic background of available cultivars (Harbinger, Herald, Angel, & Jaguar).

Powdery mildew is consistently rated highly in Queensland as the most damaging pathogen of medics (Lloyd et al, pers. comm.).

Gap/Opportunity
There is little known about the basic disease cycle (epidemiology) of the pathogen in these environments. Two resistance sources have been identified and can used for cultivar development.

This is an emerging and serious issue for livestock producers in the cereal livestock zone, where more than 5 M ha of medic pastures are based on cultivars that are highly susceptible to the disease. Incidence of the disease is increasing based on reports from growers, observations in field trials and its high ranking in the survey of agronomists. The pathogen is widely distributed. For example, in SA in 2012 infections occurred on farms on Eyre Peninsula and in trials at the Waite Institute, the Mid North and the Mallee. Infection of the susceptible cultivars was severe, with levels of leaf infection approaching 100% at flowering. The consequences of this for seed set and persistence needs to be quantified. Early infections have seen some paddocks destocked as a result of plants failing to recover from the infection. Some concerns have also been raised about the palatability of the infected plants. Improved understanding of the conditions leading to early infections is needed to enable the development of control strategies.

Preliminary assessment of resistant breeding lines indicates that large gains in legume production of about 30% are possible. The benefits of resistant cultivars will be easily seen and this should encourage good adoption. Increased levels of pasture renovation will provide the opportunity to introduce other traits (eg herbicide resistance) where they can be combined with the powdery mildew resistance.

Approaches
A 3-pronged approach is needed- 1)basic epidemiology studies to understand pathogen survival, hostrange, define pathogen races and understand triggers for early infection process, 2) short-term management strategies-fungicide and grazing, 3) genetic resistance and breeding solutions investigated.

Resources and linkages needed
There needs to be a pathology project combining epidemiology studies with management (fungicide, grazing) solutions and a breeding project, with plant pathology input to understand sources of genetic resistance and their mechanisms. The two sources of resistance (PM1 and PM2) and pathology expertise are available at SARDI.

The Risk
Low risk

Time Scale
Basic epidemiology research and management is short term (<5 years)
Breeding solution is medium term (4-7 years) with some advanced breeding material available for evaluation.

Outcome - and how measured
Short-term tools to manage powdery mildew, based on sound understanding of the pathogen and PM-resistance trait transferred to well-adapted cultivars.

5.3 Pasture pests

Identified gaps: Mites, specifically RLEM and Blue Oat Mite, were the pests most nominated as research topics. For RLEM the major issue was the emergence of insecticide resistance in WA and its anticipated transfer or development in the eastern states. The need for research was driven mainly from the mixed farming zones. The need for the development of resistant cultivars and for more effective integrated pest management rated highly.

The development of pesticide resistance in aphids and the potential for pesticide resistance to develop in other invertebrate pests also requires that a more integrated approach to management be developed. Researchers identified that selection pressures for insecticide resistance are coming from cropping systems, pasture legume seed producers, as well as pasture systems. There is a trend across industries towards “preventative” spraying, rather than monitoring and spraying when required. This is increasing the potential for resistance build up and for residue impacts in meat, affecting market access.

In the northern high rainfall areas of NSW, a better understanding of the population dynamics and biology of Blue Oat Mite was seen as a priority by interviewees. Gaps in knowledge identified were the distribution of different races of BOM and the implications for management strategies and differences in susceptibility to insecticides.

5.3.1 Proposed area of investment: optimisation of pesticide use on farm in southern Australia

The Issue
The rationale for the project is to collect information that should lead to <u>reduced use of pesticides</u>, thereby avoiding overuse, saving money, minimizing non-target effects and minimizing the risk of development and spread of pesticide resistance among pasture pests, which is an important and current issue.
The immediate aim of this work is to find out current patterns of pesticide use in pasture systems in southern Australia, including amounts of pesticide, stated target pests, timing and placement. This type of information is not available, despite hundreds of millions of dollars being spent on pest control in pastures and crops annually (Lawrence, 2009). According to Radcliffe (2002), 8,000 tonnes of active ingredient of pesticides were used annually on farms in the late 1990s and trends were towards increasing use. While most of this active ingredient was applied to crops, a significant component would also have been used to treat invertebrate pests of pastures.
This study applies to all pasture systems across southern Australia, and pasture management decisions that are made on a regular basis, with potentially large impact. Total area: up to 25 million ha of sown pastures in southern Australia (Wolfe, 2009).
NOTE: Trend figures for pesticide use are difficult to obtain, collate and interpret. Pesticide usage is thought to have increased in recent years, but this should be backed up with data. The information provided by Radcliffe (2002) refers to the late 1990s and this should be updated, since agricultural production systems have changed considerably over the intervening period.

Background

The timing and placement of pesticide treatments are important, but it is very likely that pesticides are overused, sometimes through lack of knowledge. More strategic use of pesticides is warranted for a number of reasons:

- Planning a program of pesticide use against a range of pests is likely to have a better outcome than controlling each pest separately.
- Pesticide resistance is a problem with Aphids (resistance to synthetic pyrethroids and organophosphates), RLEM (resistance to synthetic pyrethroids) and potentially in Lucerne Flea. Overuse of pesticides contributes unnecessarily to this problem.
- Reduced cost and reduced risk of residues in the environment and in agricultural produce.

Employing combinations of pest control methods (chemical pesticides alongside plant resistance, grazing management as well as chemical treatments, encouraging natural enemies) will be beneficial.

Gap/Opportunity

There is an opportunity to be more precise and strategic in the timing and placement of pesticides (saving money and time as well).

There is an opportunity for current insecticide treatments to remain effective for a longer period (reduced risk of appearance and spread of pesticide resistance).

Lastly, there is an opportunity to identify gaps in knowledge.

Approaches

A survey of insecticide use on pastures is proposed, via a combination of working with chemical companies, regional consultants, and farmer survey, also involving APVMA, possibly DAFF and ATSE (Radcliffe, 2002). Information to be collected: amount of active ingredient used, target pest(s), timing and placement.

The results of this survey need to be combined with information on the rationale for current pesticide use patterns, and how well these relate to “best practice”.

It will be beneficial to work with leading farmers who have kept good records and who implement good management practices. It will be useful to seek information from farmers who employ lower rates of pesticide usage that are nevertheless effective.

Resources and linkages needed

A suitably qualified small team is needed, to design and conduct the study. Activities: collect and assemble data, interpret data, and then draw conclusions that relate pesticide usage to pest ecology and pasture management.

Possible co-investment: GRDC (possibly adding value to the current “National Invertebrate Pest Initiative”), Dairy and Wool RDCs.

The Risk

There is a potential for lack of accuracy in collection and assembling the data (both quantitative and qualitative errors).

Deficiency, rather than a risk: collecting data on pesticide use will not by itself give information about effectiveness of pest control.

Time Scale

Short term: three to 6 months to start. Allow 12 months for the study and submission of final report.

Outcome and how measured

Survey completed, current use patterns for pesticides described, opportunities to reduce pesticide use, or to use insecticides in a more targeted way (time and space) identified.

Benefits to growers
There are wider implications of being able to reduce pesticide use:

5.3.2 Proposed area of investment: potential for biological control of key pests (aphids, lucerne flea and mites) using natural enemies in pastures.

The Issue
Aphids, lucerne flea and mites, together, are the most important invertebrate pests of legume-containing pastures in southern Australia in terms of scale, extent and frequency (see Appendix 1, Appendix 3). Taken together, aphids, lucerne flea and mites have a very broad host range among legume species. The total area potentially affected is at least 17 million ha of perennial and annual pastures containing legumes (Wolfe, 2009*) and these pests occur regularly across that area (Appendix 3). It has been estimated that Redlegged Earth Mite alone caused $130 – 200 million of production losses in improved sheep and cattle pastures (Ridsdill-Smith, 1991). Aphids, lucerne flea and mites can cause serious damage to pastures mites at germination and early seedling growth (autumn and winter) as well as during the spring growth flush. Aphids can cause seed loss of up to 90%, which can have a major impact on regeneration of pastures (Allen et al., 1989; Hopkins et al., 1994). Finally, aphids spread economically important viral diseases such as alfalfa mosaic virus (see Appendix 2).

Background
There is a substantial amount of information about the extent and severity of the problem of aphids, lucerne flea and mites as pests of pastures (see Appendix 3). There is some published scientific research information about predators of aphids (eg parasitic wasps, see Pavri and Young, 2007) and predatory mites such as snout mites which can attack lucerne flea and blue oat mite (Ireson and Webb, 1995; Umina, 2007c; Roberts et al., 2011).

Gap/Opportunity
There is an opportunity to use natural enemies of important pests more effectively, together with an opportunity to reduce pesticide use.

Training – Australia urgently needs to build capacity in pasture entomology, to maintain and improve the knowledge and skills base and to help ensure good pasture productivity in the future. In this particular case, the skill development is in relation to the ecology and control of key invertebrate pests.

Approaches
The proposal is to establish PhD studentships to conduct investigations into predators of selected aphids, lucerne flea and/or mites, and their effectiveness in pest control. There would need to be at least two separate projects, one for the study of parasitic wasps of aphids and the other for the study of predatory mites that attack pest lucerne flea and pest mites.

The study would need to focus on the biology, host range and efficacy of natural enemies and predators of pest species.

Resources and linkages needed
PhD stipends, qualified supervision.

The Risk
The risks inherent in attempting to obtain effective biological controls are inconsistency of pest control, and a slower response than is generally obtained from chemical control methods.

There is a risk of the selected student not completing project (this can normally be managed).

Time Scale
Projects: short term, i.e. 3 – 4 years to complete. Time scale to use results on farm: medium to long.

Outcome and how measured
Potential biological controls for aphids, lucerne flea or mites in pastures.

5.3.3 Proposed area of investment: control of pasture scarabs and corbies in high rainfall areas

The Issue
Red-headed Cockchafers (RHC) cause serious damage to both perennial pastures and rotational crop / pasture systems in southeastern Australia. RHC have a broad host range that includes both legumes and grasses. RHC is a known, regular, serious pest but its control is at present an intractable problem. The importance of Yellowheaded Cockchafer (YHC) appears to be increasing but the extent and severity of the problem is at present not well-documented (see Appendix 1). Chemical control of both RHC and YHC is difficult and there are no effective control options other than growing RHC-tolerant varieties or species of pasture grass. The area of pasture seriously affected by RHC and YHC appears to have increased dramatically to 160,000 ha in the past 20 years in Tasmania alone (Appendix 4).

Losses caused by RHC in Victoria in 1993 were estimated to be $1 million and in a separate study, losses of $100/ha for lamb production and $500/ha for dairy pastures (see Berg 2008, referenced in Appendix 4).

Pasture scarabs such as *Sericesthis geminata* can be a serious problem in Northern NSW (see Appendix4).

Corbies (*Oncopera* species) are routinely nominated as one of the most serious pests of pastures in Tasmania and the wetter regions of Victoria (Appendix 1, Appendix 3). Current control methods use Chloropyriphos which is currently under review by the AVMPA. There is a need to develop alternative control methods

Scale: these insect pests affect mainly permanent pastures in higher rainfall areas of SE Australia. The area of permanent pasture in this part of Australia was approx 3 million ha in 1997 (Dear and Ewing, 2008, cited in Appendix 4). In addition, pastures of Northern NSW and SE Queensland can also be seriously affected by pasture scarabs (total area likely to be several million ha).

Background

The life cycle of RHC occurs almost entirely underground, so it is difficult to control with chemical pesticides. 'Victoca' ryegrass was selected for tolerance to RHC and is a popular cultivar in the drier parts of Tasmania. Cocksfoot and brome can also be grown as RHC-tolerant pasture grasses. The fungus *Metarhizium anisopliae* has been used with some good results but at present the commercial product (BioGreen™) is not available.

The larvae of YHC feed on a wide range of pasture plants and chemical control is difficult, as noted for RHC. While fungal diseases like *Metarhizium* attack the larvae in the soil, no effective biological controls have been reported for YHC. Grazing intensity influences populations of these root feeding insects but this has not been used to develop control methods.

Chemical control of Corbies and Winter Corbies can be effective after grazing, but depends on timing as a critical factor. A number of fungal species can cause disease in corbies and two species of entomopathogenic nematode were trialled against Winter Corbie in turf, with very good results (see Appendix 3). There do not seem to have been field evaluations of biocontrol agents in pastures.

Gap/Opportunity

Control measures are urgently needed for serious Scarab pests of pastures in high rainfall areas that are difficult to control. Research into improved ways of reducing damage caused by Corbies is also required.

Given that these insect pest problems affect the higher rainfall areas in general, co-investment from Dairy and Wool RDCs could be sought.

Approaches

RHC: a suggested approach is to fund a PhD project, possibly looking at conditions that favour the plant versus conditions that favour the insect. Light trapping can be used to catch adults, which are night fliers. Research questions include: when and why should insecticide be used? The results need to be linked to pasture management practices.

YHC: information needs to be collected about the importance of this emerging pest, including geographic extent and severity of damage caused.

Corbies: a similar PhD program to the RHC project should be designed for research on the biology and control of Corbies and Winter Corbies.

Resources and linkages needed

PhD student(s) and suitably trained entomologist(s), with expertise in these pests as well as research methodology based in SE Australia (Vic, Tas or SE of SA). Northern NSW can also be considered as a venue for research on pasture scarabs. Links to graziers and possibly to chemical and seed companies will be useful.

The Risk

With suitable supervision this work should have a high chance of success.

Time Scale

Short to medium term.

Outcome and how measured
New control measures for pasture scarabs and/or corbies, or new integrated control combinations.

5.3.4 Proposed area of investment: breeding for resistance to the new biotype of bluegreen aphid in pasture legumes

The Issue
Serious damage has recently been caused to pasture legumes by a new biotype of Bluegreen Aphid (BGA) which has overcome plant resistance (Humphries et al., 2010, see Appendix 3). This new biotype of BGA is a serious threat to clover, medic and lucerne-based pastures and could potentially affect up to 17 million ha of legume-containing pastures across southern Australia, including both perennial and annual pastures. Significant damage from aphids can also lead to large losses in seed production, which can in turn reduce pasture regeneration. The role of BGA as a vector of virus diseases also needs to be taken into account.

Background
The apparently accidental introduction of BGA and SAA into Australia in the 1970s caused widespread, serious economic losses in legume pastures (as well as in susceptible crops). The selection and breeding of aphid-resistant cultivars of pasture legumes has resulted in better survival and productivity. However, aphids can develop new biotypes which overcome plant resistance, especially if this is single gene resistance. The recent appearance of a new biotype of BGA that has overcome plant resistance (Humphries et al., 2010) could have very damaging consequences.

There is apparently some resistance to the new biotype of BGA in legume germplasm at SARDI which can be utilised as a starting point for a breeding program.

Gap/Opportunity
The problem has been identified, and the potential for serious effects on pasture production is clear.

Approaches
Plant breeding, utilizing resistance that is present in germplasm held by SARDI. This work could be done alongside existing pasture legume breeding programs (medic, sub-clover, lucerne) by appropriate groups of scientists.

Resources and linkages needed
The project requires plant breeders who have a focus on pasture legumes and have access to appropriate facilities and suitable germplasm.

The Risk
Low risk if there is already some resistance in existing plant germplasm accessions.

Time Scale
Up to 12 months to start, medium to long term to finish: 10 years.

Outcome and how measured
Resistance to the new biotype of BGA incorporated into commercial varieties of one or more of the following: medic, lucerne, sub clover.

5.3.5 Proposed area of investment: assessment of the impact of weevils on pasture production in southern Australia

The Issue

An assessment of the importance of weevils as pests in pasture production is urgently needed. White fringed Weevil (WFW) appears to be increasing in distribution and importance in south eastern Australia (see Appendix 1, Appendix 3). Sitona Weevil attacks a wide range of legumes and can interfere with N fixation via damage to root nodules, but its overall importance to pasture production is a matter of debate. In lucerne production there are two other weevils to consider: Small Lucerne Weevil and Broadback Weevil. In medic pasture / crop rotations, a new weevil (*Mandalotus* spp, Rubble Bug) appears to be an increasing pest (Appendix 3). While *Mandalotus* is not considered to be a serious problem in the pasture phase at present, its impact should be monitored.

An assessment is required in order to decide the need, and best strategies, for pest control and R&D into the weevil problem.

<u>Scope and extent</u>: the weevil species mentioned here attack a range of common pasture legumes, so the total area of pasture potentially affected is very large, probably several million hectares (including the permanent pastures of SE Australia as well as a proportion of the lucerne growing area of NSW, but excluding the medic pasture area).

Background

Sitona Weevil and its impact were researched reasonably well in southern Australia in the period 1970 - 1990. Other weevils (WFW, *Mandalotus*) appear to be increasing in importance according to survey and field research results (Appendix 1, Appendix 3). Because weevils are difficult pests to control, it is important to assess the pest status of the various weevil species in present day grazing systems.

Gap/Opportunity

Damage to permanent and annual pastures caused by weevils appear to be increasing & weevils are difficult to control. Currently the main issue is that the extent of the problem and actual impacts of weevils are not clear. This gap needs to be addressed.

Approaches

The proposal is to undertake survey work specifically directed at assessing pasture damage due to weevils. This can be done by scientists or trained district agronomists working with consultants and growers.

Reference should be made to reports and international scientific literature on current control measures in Australia and elsewhere.

Resources and linkages needed

The work should be done by suitably trained entomologist(s), with expertise in working with these pests, as well as a good knowledge of research, survey and sampling methodology. The work should be done in liaison with growers and possibly also with chemical and seed companies.

The Risk
Chances of success in this project are very good.

Time Scale
Immediate short term: 12 to 18 months.

Outcome and how measured
Assessment of the importance of weevils as emerging pests and collated information about control options or suggestions of R&D into control measures; report completed.

Benefits
Assessment of relative importance and pest status of weevils should allow informed decisions to be made about pest control.

6. Appendix 2

Opinion Survey of Pasture Agronomists

6.1 Background

A phone survey was done in October and September 2011 to gauge the understanding of pasture health issues in the field. Because of the episodic nature of some pests and diseases those selected to be interviewed generally had extensive experience of operating as agronomists. Farmers were not specifically targeted because of the assumption that they would mainly report on localised issues that affected them personally and would not be able to provide a broader regional perspective. Further it was considered that most producers would not be able to identify many of the less obvious pests and diseases. However, one quarter of the agronomists interviewed were also practicing farmers.

Almost 80% of those interviewed had been in their current role longer than 5 years. Of this cohort, two thirds had had experience greater than 5 years in related employment. This length of experience indicates that those interviewed had significant experience over a range of seasons and provides confidence in the results of the survey

The geographic distribution of those interviewed is shown in Table 1. The numbers in each zone roughly reflect the size and importance of that zone. Some of those interviewed operated across state borders which accounts for the total number being greater than 41, in the Table below.

Table 1 Number of agronomists interviewed by state and by agro-ecological zone

State	Agro-ecological Zone			
	Wet Temperate Coast	Temperate Highlands	Temperate Slopes & Plains	Sub-tropical Slopes & Plains
NSW	1	8	8	2
Victoria	5	3	4	
Tasmania	3	3		
South Australia	3		7	
Western	2		8	

Australia				
Total	13	14	27	2

The agronomists were interviewed by phone and asked to provide information on:

- Pest and diseases of pastures and lucerne at establishment
- Pests and diseases of established :
    - Annual clovers
    - Perennial clovers
    - Annual medics
    - Lucerne
    - Alternative legumes (eg Serradella and Biserrula)
    - Grass pastures

Those interviewed were asked to provide qualitative information about the geographical and temporal distribution of the pest/disease and the site specific economic impact of the pest/disease.

The categories and ratings for these three criteria were as follows:

Table 2 Rating scales used for the assessment of the significance of pest and diseases

Geographic distribution		**Frequency of occurrence**		**Economic Impact**	
Category	**Rating**	**Category**	**Rating**	**Category**	**Rating**
Isolated	1	Less frequent than 1 in 5 years	1	Minor	1
Regional	2	1 in 5 years	2	Minor to moderate	2
Widespread	3	1 in 4 years	3	Moderate	3
		1 in 3 years	4	Moderate to severe	4
		1 in 2 years	5	Severe	5

		Annually	6		

In order to provide a relative ranking of the importance of each of the pests and diseases, the ratings for each of the criteria were multiplied together. Thus a widespread problem that occurred annually and created severe economic impact would have a combined rating of 90.

Interviewees were also asked to provide an opinion about the effectiveness of rhizobia in their district.

They were also asked to nominate, with justification, key areas for research and development in pests and diseases and in rhizobial function.

The validity of the responses provided by the interviewees is dependent on the ability of agronomist/extension officer to recognise either the pest or the damage it causes and the symptoms of specific diseases.

To gauge individual skill levels, interviewees were asked if they could confidently identify a range of pests and diseases and/or the damage they caused. The results of this component of the survey are discussed in the “Capacity Building” section. They were also asked to provide opinions on the adequacy and availability of information on pests and diseases of pastures and lucerne.

6.2 Pest and Disease Recognition

The recognition of pest and diseases was asked at the end of the survey so as not to bias the results. However it is instructive to consider the results of these questions at this stage as a means of putting the results of the pests and diseases for individual situations into context. Table 3 shows the recognition level for pests, Table 4 the recognition level for pasture diseases and Table 5 the recognition level for lucerne diseases

Table 3 Number of interviewees who indicated that they could confidently recognise the following pests or the damage caused by the pest

Pest	**No**	**% Recognise**
African Black Beetle	21	53%
Argentinian Stem Weevil	3	8%
Armyworms	35	88%
Black Field Crickets	29	73%
Blackheaded Cockchafers	27	68%
Blue Green Aphid	34	85%
Blue Oat Mite	31	78%
Bryobia Mite	23	58%
Clover Nematode (White Clover)	4	10%
Lucerne Flea	36	90%
Lucerne Leaf Roller	19	48%

Oncopera Spp. (Corbies)	9	23%
Oxycanus Grass Grub	4	10%
Pasture Root Aphids	15	38%
Pasture Webworm	29	73%
Pea Aphid	31	78%
Redheaded Cockchafers	30	75%
Red-Legged Earthmite	39	98%
Root Knot Nematode (Sub Clover)	9	23%
Root Lesion Nematode (Medics)	14	35%
Sitona Weevil	32	80%
Slugs	38	95%
Snails	36	90%
Spotted Alfalfa Aphid	30	75%
Stem Nematode (White Clover)	4	10%
White Fringed Weevil	20	50%

There was generally a reasonable recognition of pests. The exceptions were the ability to identify underground pests (eg nematodes, root aphids) and the recognition of geographically specific pests such as corbies.

Table 4 Number of interviewees who indicated that they could confidently recognise the following diseases of pastures

Disease	**No**	**% Recognise**
Aphanomyces (Sub Clover)	2	5%
Barley Yellow Dwarf Virus (Grasses)	24	60%
Brown Leaf Spot (Medics)	12	30%
Clover Red Leaf Virus	21	53%
Clover Stunt Virus	15	38%
Crown Rust (Grasses)	21	53%
Ergot (Grasses)	27	68%
Fusarium Root Rot	17	43%
Kabatiella (Sub Clover)	16	40%
Phoma Stem Rot (Medics)	7	18%
Phytophthora clandestina	27	68%
Powdery Mildew	37	93%
Pythium Root Rot	17	43%
Rhizoctonia	20	50%
White Clover Mosaic Virus	11	28%
White Clover Rot (Sclerotinia)	10	25%

Table 5. Number of interviewees who indicated that they could confidently recognise the following diseases of pastures

Disease	No	% Recognise
Acrocalymma Crown Rot	6	15%
Alfalfa Mosaic Virus	18	45%
Anthracnose (Colletotricum)	13	33%
Aphanomyces	7	18%
Fusarium Wilt	19	48%
Leptosphaerulina	9	23%
Lucerne Yellows	17	43%
Phoma	9	23%
Phytophthora Root Rot	19	48%
Pseudopeziza Leaf Spot	12	30%
Pythium Root Rot	18	45%
Rhizoctonia Root Rot	17	43%
Stagonospora Crown And Root Rot	11	28%
Stemphylium Leaf Spot	9	23%
Uromyces (Rusts)	22	55%

In contrast to the recognition level for pests, the recognition level for both pasture and lucerne diseases was poor. There was a poor ability to differentiate between the various root disease fungi and the viral diseases.

6.2.1 Adequacy and availability of pest and disease information

The availability and adequacy of pest and disease information of pastures and lucerne rated between 2.4 and 2.6 on a scale of 1-4

It was considered that there was good information around, but that much of it was resident in individuals or organizations and therefore was vulnerable. This applied in particular to State agencies. Gaps in information related to the currency of control methods, epidemiology, integrated management and economics of control.

From a farmer perspective, the agronomists thought that the availability of data was seen to be inadequate, as was the training of new agronomists. There were a number of suggestions that MLA follow the GRDC model in the provision of ute guides, grower updates and support for an organisation such as CESAR to provide disease and pest information for the feedbase. As a further indication of the need for information two thirds of those interviewed said that they would attend pest and disease update workshops with a further 20% attending depending on content.

The need for a pest and disease identification services was highlighted because of the poor ability of many agronomists and producers to recognise a number of the pests and diseases.

6.3 Pests and diseases

The results for the pests and diseases of pastures and Lucerne during establishment, and for each of the plant species in established swards, are shown below**. In each of the figures, the number of respondents nominating the particular pest is shown on the horizontal axis and the average rating of significance shown on the vertical access**. Only those pests and diseases which were mentioned by respondents have been included in the graphs.

6.3.1 Pest and Disease at Establishment

6.3.1.1 Pastures

The pests nominated as affecting pasture establishment are shown in Figure 5.

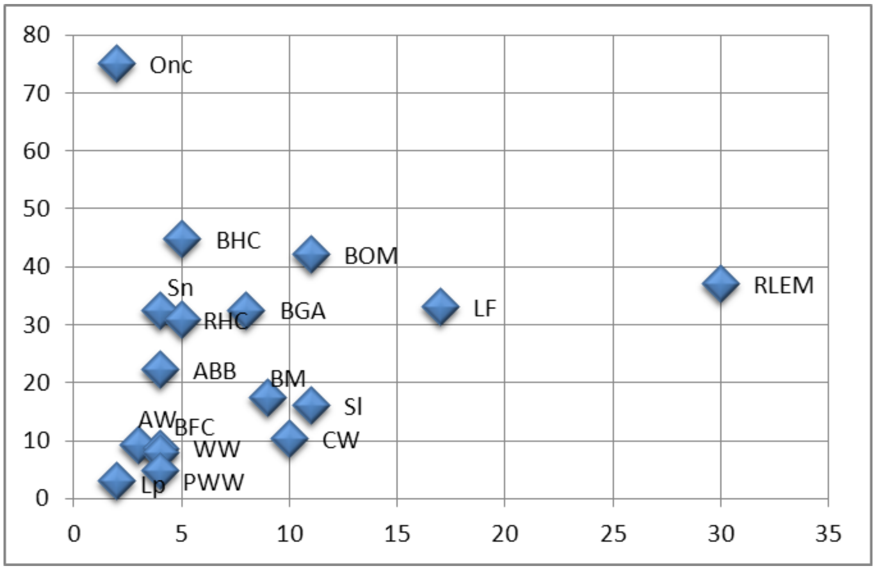


Figure 5 Average rankings of pests at pasture establishment and the number of times nominated by interviewees

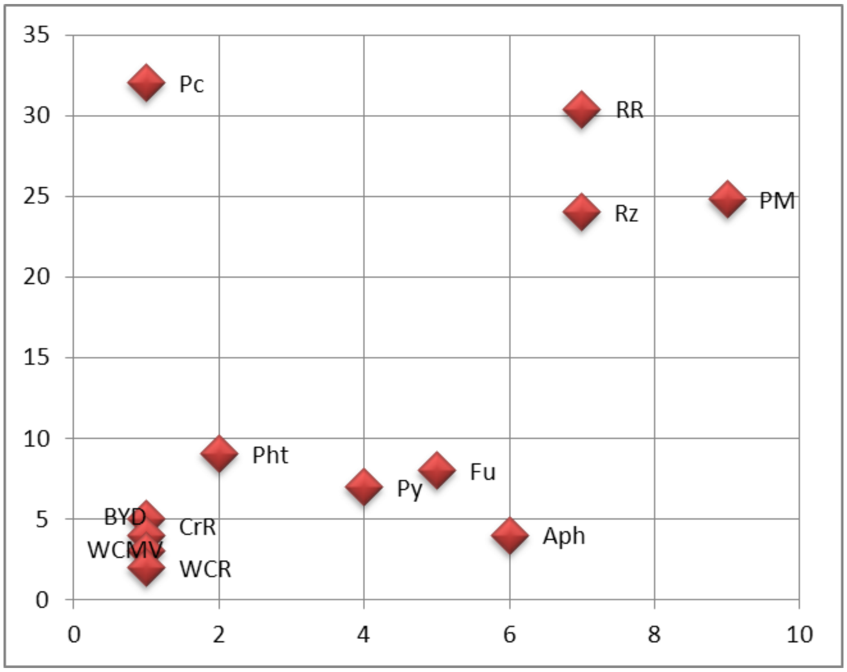
African Black Beetle.	ABB
Armyworms.	AW
Black Field Crickets.	BFC
Blackheaded Cockchafers.	BHC
Blue Green Aphid.	BGA
Blue Oat Mite.	BOM
Bryobia Mite.	BM
Cutworms	CW
Loopers	Lp
Lucerne Flea.	LF
Oncopera spp. (Corbies).	Onc
Pasture Webworm.	PWW
Redheaded Cockchafers.	RHC
Red-Legged Earthmite.	RLEM
Slugs.	Sl
Snails.	Sn
Wireworms -	WW

The highest ranking pest of establishing pastures was Oncopera spp. These nominations were confined to Tasmania.

Mites rated highly overall as pests of establishing pastures Red Legged Earthmite (RLEM) was seen as the most widely dispersed pest, being nominated in those areas south of Dubbo and across to Western Australia. Blue Oat Mite (BOM) ranked more highly in northern NSW and in those areas where there had been regular treatment for RLEM which had changed to mite population towards BOM. Bryobia mite was reported from WA and NSW, but the relatively poor recognition of mites may indicate that it is underreported. There was some divergence of opinion of the economic importance of the mite. If untreated damage was considered significant, however they were considered controllable in most pasture establishment situations although reports from Western Australia indicated the emergence of resistance by RLEM to synthetic pyrethroids.

Lucerne Flea (LF) was the second most nominated pest and ranked relatively highly in relation to its significance.

A number of pests (slugs, Red Headed Cockchafers and Blackheaded Cockchafers) have increased in significance as a result of the use of direct drilling for pasture establishment.

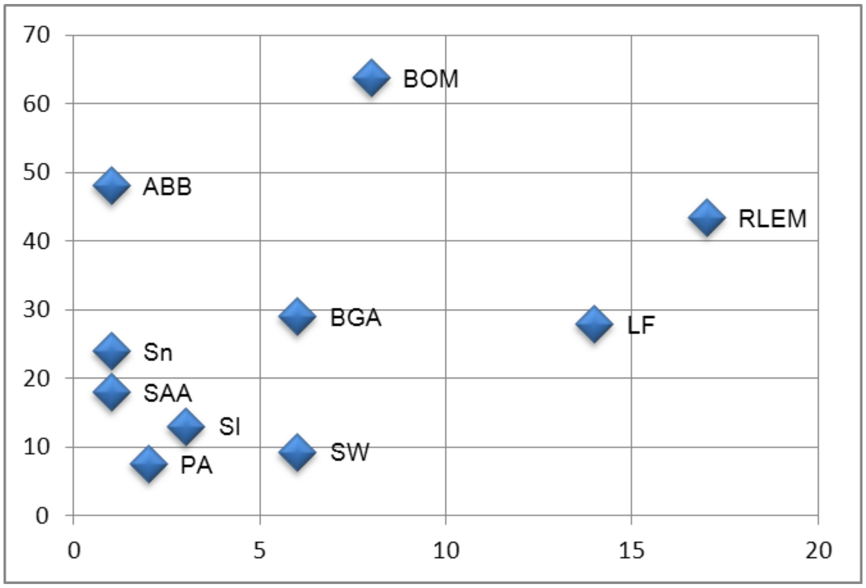


Aphanomyces (Sub Clover) -	Aph
Barley Yellow Dwarf Virus	BYD
Brown Leaf Spot (Medics)On	
Crown Rust (Grasses)	CrR
Fusarium Root Rot	Fu
Phoma Stem Rot (Medics)	Ph
Phytophthora clandestina	Pc
Powdery Mildew	PM
Pythium Root Rot	Py
Rhizoctonia	Rz
Root Rots (Unspecified)	RR
White Clover Mosaic Virus -	WCMV
White Clover Rot (Sclerotinia)	WCR

Figure 6 Average rankings of diseases at pasture establishment and the number of times the disease was nominated by interviewees

Compared with pests, diseases of establishing pastures rated much lower in significance. Root diseases were the most common problem reported. However, it was evident that a number of interviewees considered that root diseases were a complex of fungi and/or that they were unable to differentiate between pathogens. Further it was also clear that fungal damage to emerging seedlings was a factor that was not recognised or assessed by many agronomists.

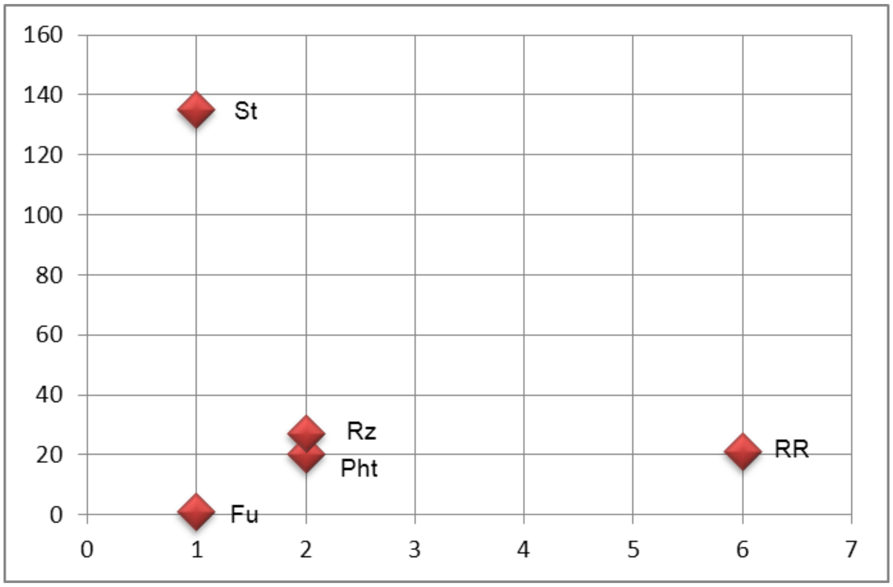
Lucerne



African Black Beetle.	ABB
Blue Green Aphid.	BGA
Blue Oat Mite.	BOM
Lucerne Flea.	LF
Pea Aphid.	PA
Red-legged Earthmite.	RLEM
Sitona Weevil.	SW
Slugs.	Sl
Snails.	Sn
Spotted Alfalfa Aphid	SAA

Figure 7 Average rankings of pests at lucerne establishment and the number of times the pest was nominated by interviewees

As with establishing pastures, RLEM BOM and LF were the most nominated pests of establishing lucerne. African Black Beetle was severe in localised areas of the southern coast of NSW. Again most pests could be managed with insecticides or avoided by altering the time of sowing, etc. (eg Blue Green Aphid - BGA)



Common Leaf Spot	CLS
Fusarium Wilt	Fu
Phytophthora Root Rot	Pht
Rhizoctonia Root and Stem Canker	Rz
Root Rots unspecified	RR
Stagonospora Crown and Root Rot	St

Figure 8 Average rankings of diseases at lucerne establishment and the number of times the disease was nominated by interviewees

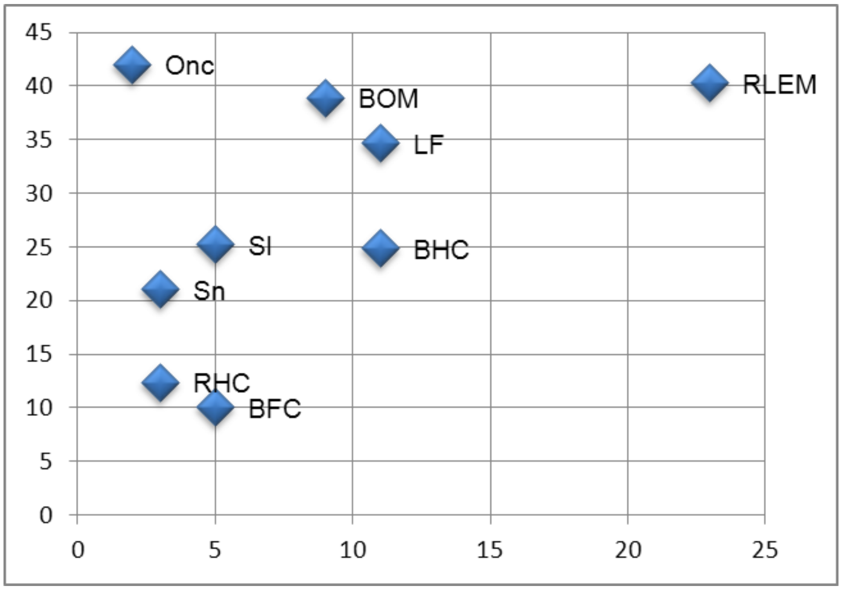
There was very little recognition of lucerne diseases affecting establishment and it was believed that breeding for disease resistance had most of the disease issues under control. There was a strong need to continue this work.

Some agronomists were able to nominate specific diseases but, as with diseases of establishing pasture legumes, agronomists tended to consider root rots as a disease complex.

6.3.2 Pests and Diseases of Established Pastures

6.3.2.1 Annual Clovers

The pests and diseases of annual clovers related mainly to subterranean clover. There were minimal or no nominations of pests or diseases specific to the other annual clovers (ie Balansa, Globe, Persian, etc.)

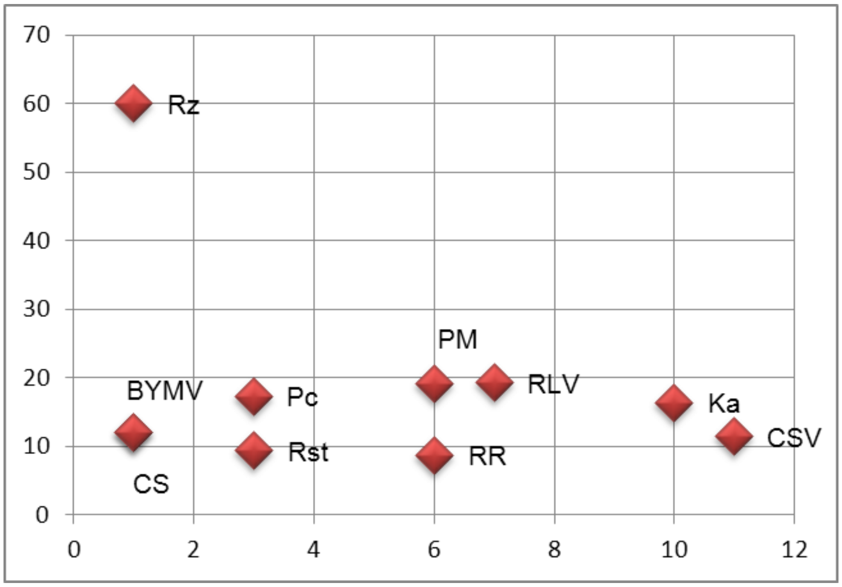


Black Field Crickets.	BFC
Blackheaded Cockchafers.	BHC
Blue Oat Mite.	BOM
Lucerne Flea.	LF
Oncopera Spp. (Corbies).	Onc
Redheaded Cockchafers.	RHC
Red-Legged Earthmite.	RLEM
Slugs.	Sl
Snails.	Sn

Figure 9 Average rankings of pests of established annual clover pastures and the number of times the disease was nominated by interviewees

The major pests of high impact were RLEM, LF, BOM and Oncopera. Again RLEM was the most nominated species. Oncopera were significant in Tasmania.

Blackheaded Cockchafers were highly damaging pasture pests when they occurred, but rated at a low level because of the episodic nature of infestations



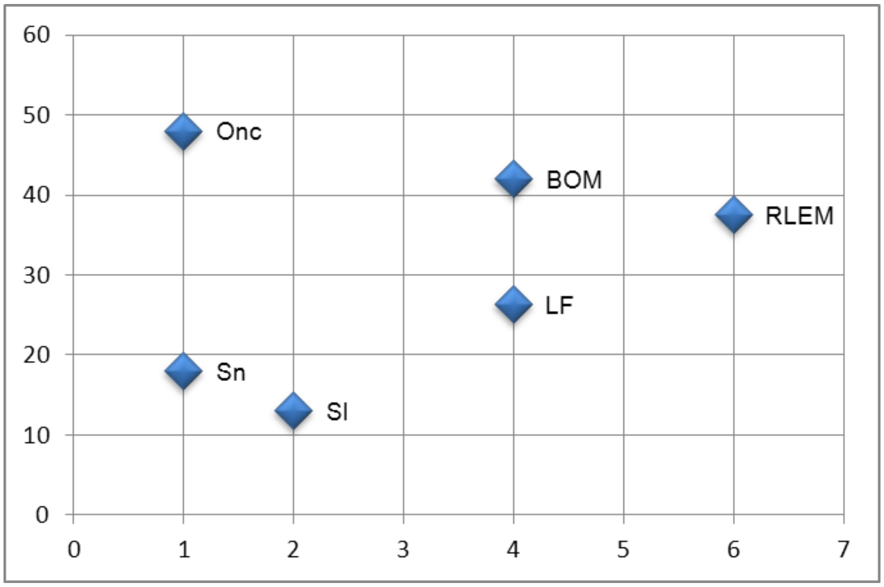
Bean Yellow Mosaic Virus.	BYMV
Clover Red Leaf Virus.	RLV
Clover Stunt Virus.	CSV
Kabatiella (Clover Scorch).	Ka
Phytophthora clandestina.	Pc
Powdery Mildew.	PM
Pythium.	Py
Rhizoctonia.	Rz
Root Rots (Unspecified).	RR
Rusts.	Rst

Figure 10 Average rankings of diseases of annual clover pastures and the number of times the disease was nominated by interviewees

As with diseases in the above categories, there were few issues reported and those that were, rated relatively lowly. However, it was clear that there was a lack of confidence by a number of those interviewed in being able to distinguish between the various viruses and also the various fungal diseases. Mention was made of previous surveys that had identified widespread clover viruses, but that their impacts appeared minimal.

6.3.3 Pests and Diseases of Established Perennial Clovers

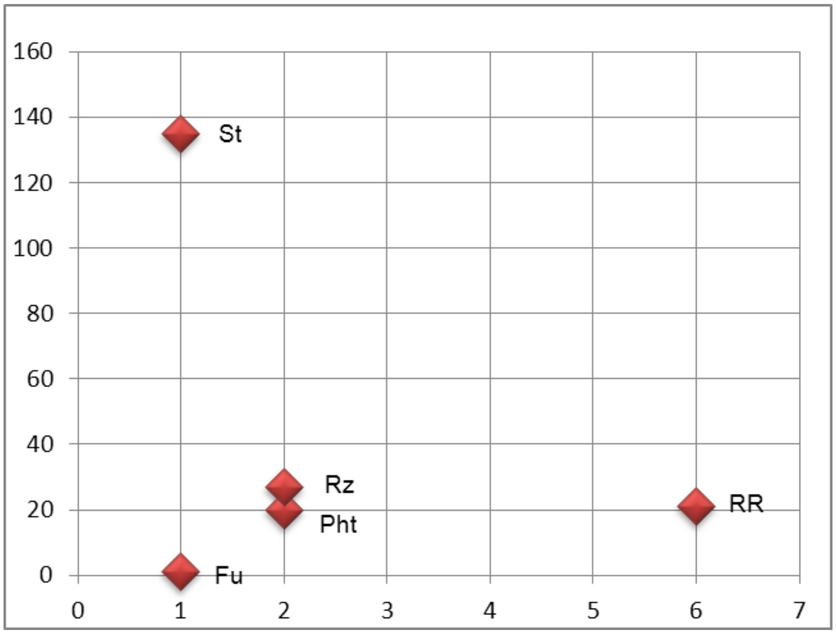
The main perennial clover considered was white clover. There were minimal references to other perennial clovers, such as red clover and strawberry clover. White clover as a component of dryland pastures is confined to the very high rainfall areas of Tasmania and Victoria and areas of the Temperate Highlands of northern NSW. White clover in irrigated areas was not considered.



Blue Oat Mite	BOM
Lucerne Flea	LF
Oncopera spp. (Corbies)	Onc
Red-legged Earthmite	RLEM
Slugs	Sl
Snails	Sn

Figure 11 Average rankings of pests of established perennial clovers and the number of times the pest was nominated by interviewees

The restricted distribution of white clover resulted in few nominations of pests of white clover. Again, RLEM was the most significant pest, but it was clear from the knowledge survey that a number of agronomists were unfamiliar with the damage caused by RLEM on white clover and may have been under reporting its impact..



Common Leaf Spot	CLS
Phytophthora Root Rot	Pht
Rhizoctonia Root and Stem Canker	Rz
Fusarium Wilt	Fu
Root Rots unspecified	RR

Figure 12 Average rankings of diseases of established perennial clovers and the number of times the disease was nominated by interviewees

Diseases were seen to be of little significance in perennial legumes. As indicated above, the low numbers of nominations is a result of the lower incidence of perennial clover species and the inclination of agronomists to look for and their ability recognise specific diseases.

6.3.4 Pests and Diseases of Established Annual Medics

Blue Green Aphid.	BGA
Blue Oat Mite.	BOM
Lucerne Flea.	LF
Red-legged Earthmite.	RLEM
Root Lesion Nematode (Pratylenchus).	RLN
Snails.	Sn
Spotted Alfalfa Aphid.	SAA

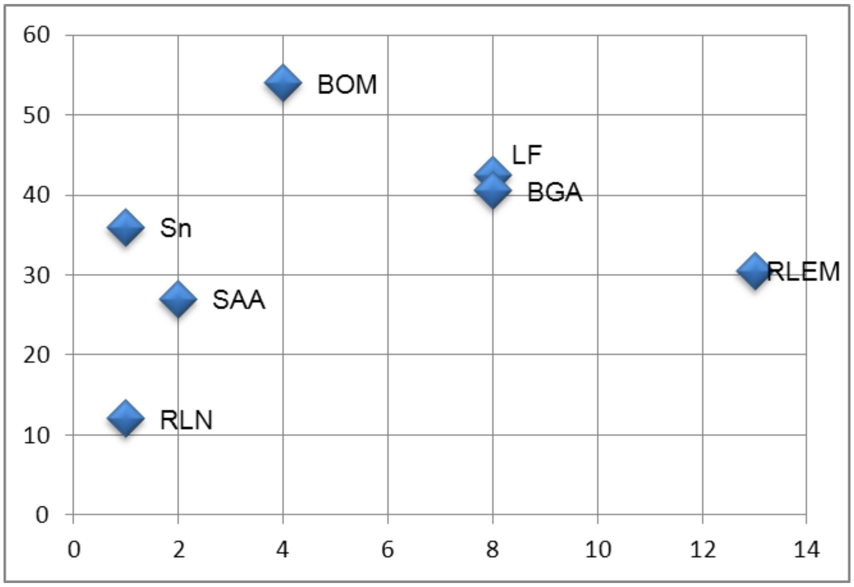
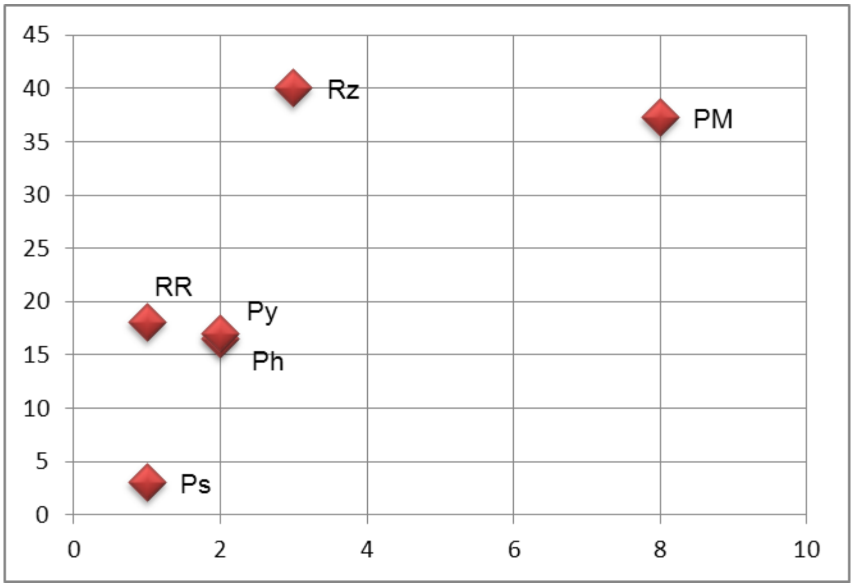


Figure 13 Average rankings of pests of established annual medic pastures and the number of times the disease was nominated by interviewees

Not surprisingly, Red Legged Earth Mite, Lucerne Flea and Blue Green Aphid were the main pests

In contrast to some comments some doubt was expressed in NSW about the significance of the Root Lesion Nematode (RLN) as there did not appear to be a growth benefit from sowing resistant cultivars in soils identified as having RLN present.

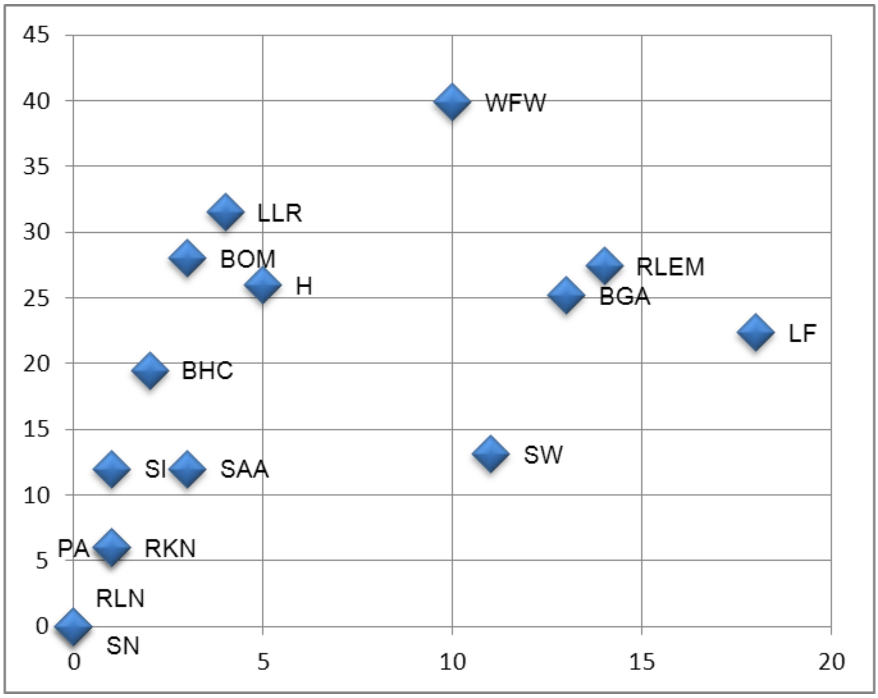


Phoma.	Ph
Powdery Mildew.	PM
Pseudopeziza.	Ps
Pythium Root Rot.	Py
Rhizoctonia Root Rot.	Rz
Unspecified Root Rots.	RR

Figure 14 Average rankings of diseases of annual medic pastures and the number of times the disease was nominated by interviewees

Powdery Mildew rated as an important and emerging disease in the cropping zones of South Australia possibly as a result of recent wet seasons. Rhizoctonia was nominated as a problem by two interviewees in SA and one in NSW but the assessment of significance varied considerably. All other diseases nominated were minor.

6.3.5 Pests and Diseases of Established Lucerne

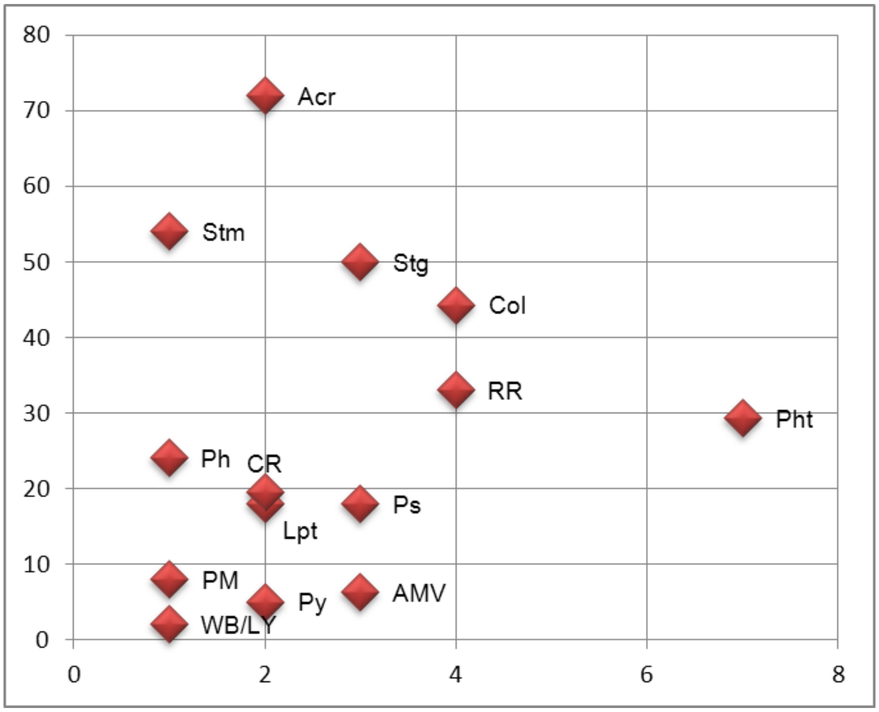


Blackheaded Cockchafer	BHC
Blue Green Aphid.	BGA
Blue Oat Mite.	BOM
Heliothis.	H
Lucerne Flea.	LF
Lucerne Leaf Roller.	LLR
Pea Aphid.	PA
Red-Legged Earthmite.	RLEM
Root Knot Nematode.	RKN
Root Lesion Nematode.	RLN
Sitona Weevil.	SW
Slugs.	Sl
Spotted Alfalfa Aphid.	SAA
Stem Nematode.	SN
White Fringed Weevil.	WFW

Figure 15 Average rankings of pests of established lucerne stands and the number of times the disease was nominated by interviewees

Those pests which rated highly with nominations of 10 or more by those interviewed were White Fringed Weevil, BGA RLEM and LF.

The most highly rating pest was White Fringed Weevil. This was seen to be an intractable problem in established lucerne growing areas from northern NSW through to Gippsland. There was a belief that an effective pathogenic nematode was released by Victorian DPI in the 1980s but this has not been confirmed. Sitona weevil generally rated low, but higher ratings for this pest were given by three agronomists, one each in Southern Slopes of NSW, the Sub-tropical Slopes and Plains of northern NSW and one from South Australia



Acrocalymma (Crown Rot).	Acr
Alfalfa Mosaic Virus	AMV
Colletotricum (Anthracnose).	Col
Crown Rots unspecified	CR
Leptosphaerulina.	Lpt
Phoma	Ph
Phytophthora Root Rot.	Pht
Powdery Mildew	PM
Pseudopeziza Leaf Spot	Ps
Pythium.	Py
Root Rots unspecified	RR
Stagonospora Crown and Root Rot.	Stg
Stemphylium Leaf Spot	Stm
Witches Broom/Lucerne Yellows	WB/LY

Figure 16 Average rankings of diseases of established lucerne stands and the number of times the disease was nominated by interviewees

Root rots were the most commonly nominated diseases of established lucerne. However, consistent with the incidence and identification of pathogens in other species, very few agronomists were able to nominate any specific disease pathogens. While this may be due to a lack of diseases, there was poor recognition of lucerne diseases by agronomists (see above) with only one disease (leaf rust – a disease with obvious symptoms) achieving a greater than 50% recognition rate.

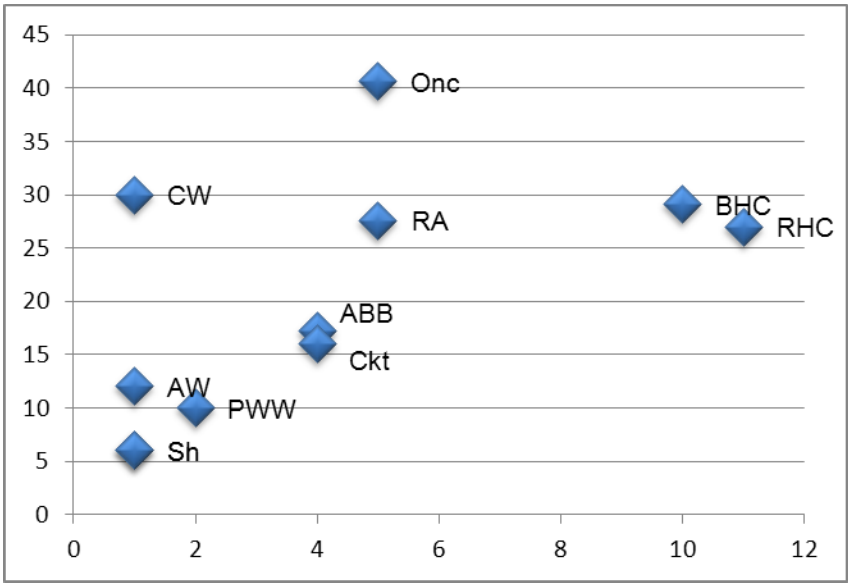
6.3.6 Pests and Diseases of Established Alternate Legumes

The only two alternate legumes of significance nominated were Biserrula and Serradella spp. Apart from Western Australia, the only other significant area of these species was on the Sub-tropical Slopes and Plains in northern NSW. However there were isolated areas of these species in other areas but were rated very lowly in terms of pest significance.

The only pests nominated as causing damage were RLEM, BGA and BOM. These nominations came from NSW. No agronomist from Western Australia identified specific pests of either of these species.

No diseases were nominated for Serradella or Biserrula by those interviewed. The only mention of a significant disease of alternate legumes was brown leaf spot of vetch in central NSW

6.3.7 Pests and Diseases of Established Grass Pastures

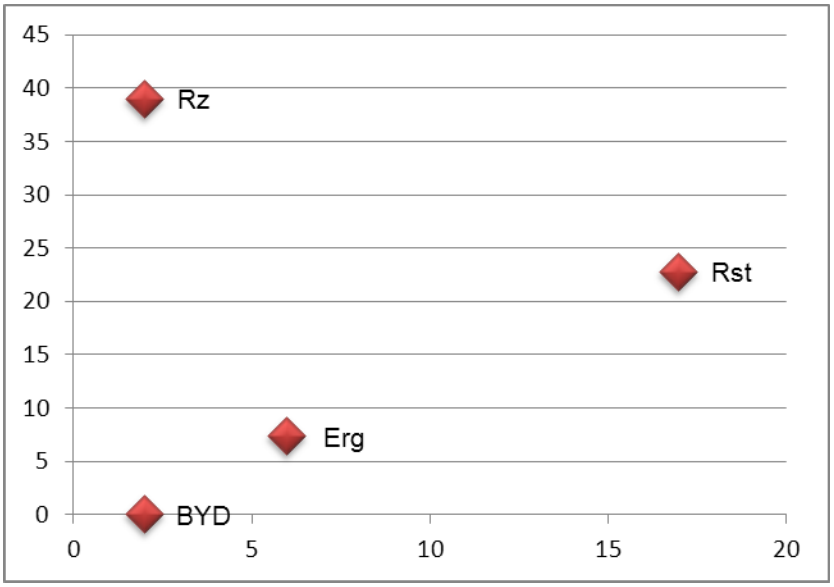


African Black Beetle	ABB
Armyworm	AW
Blackheaded cockchafers	BHC
Corbies	Onc
Crickets	Ckt
Cutworm	CW
Pasture Webworm	PWW
Red Headed Cockchafers	RHC
Root Aphids	RA
Slugs	Sl
Snails	Sn

Figure 17 Average rankings of pests of established grass pastures and the number of times the disease was nominated by interviewees

Cockchafers were seen to be the major pests of established pastures. The Red Headed Cockchafers were nominated throughout the Temperate Highlands in Tasmania and NSW and the Temperate Highlands and Wet Temperate Coast in Victoria. The problem was seen to be increasing in Tasmania with the development of overlapping generations. The incidence of Yellow Headed Cockchafers was seen to be increasing in the Monaro and Tasmania. Again Corbies were a major pest in Tasmania but were also nominated from the high rainfall areas of western Victoria.

Other pests nominated as potential or undefined issues were root aphids and root weevils – these were not given a rating and do not appear on the graph.



Barley Yellow Dwarf Virus	BYD
Pythium	Py
Rhizoctonia	Rz
Rusts	Rst

Figure 18 Average rankings of diseases of established grass pastures and the number of times the disease was nominated by interviewees

Very few diseases of grasses were nominated. However given the poor recognition of pasture diseases by interviewees, it is likely that there is underreporting of disease issues.

The most commonly identified disease was rust, particularly on ryegrass. Rhizoctonia reported as an issue in the Wet Temperate Coast of WA and the Monaro.

The one outstanding issue was the impact of Barley Yellow Dwarf Virus (BYDV) on pasture grasses. Mention was made of early work in the 1960-70s which reportedly showed impacts of BYDV on pasture grass production, but which does not appear to have been followed up. (Hence BYDV is shown on the graph but without a rating)

6.4 Rhizobial function

Interviewees were asked how they would rate the effectiveness of nodulation/rhizobial function in established stands for a range of legume species. On average, respondents indicated that they believed that nodulation was moderately effective to highly effective. When asked their reasons for such a statement, most admitted that they had no evidence or that they assumed because the seed was inoculated and/or that pink nodules were present, effective nodulation had taken place. Some of those interviewed on the other hand, were aware of evidence that indicated poor rhizobial function.

However there was considerable concern expressed about the level of viable rhizobia on inoculated seed.

6.4.1 Rhizobial/nodulation research issues

There was general concern about the performance of the legume/rhizobial relationship in terms of the longer term impact on productivity and the supply of nitrogen ton both pastures and crops.

The research issues identified by consultants can be grouped into five broad categories:

- Rhizobial effectiveness in the field and
- Rhizobial survival in the field.
- Effectiveness of current seed coatings
- The development of new strains
- The impact of herbicides on rhizobial function in mixed farming systems

The most commonly nominated issue was to understand and be able to measure the effectiveness of rhizobial function in the field. In related topics, the survival of rhizobia in the field as influenced by either acid soil conditions, cropping rotations and the use of herbicides also rated very highly.

The continued development of new strains was encouraged and the ability to introduce new strains into existing swards or into swards where rhizobia were absent or in low levels was also mentioned.

There was a high level of suspicion about the effectiveness of rhizobial seed coatings. It was unclear to those who were interviewed whether this was an issue of quality control somewhere along the supply chain or if the techniques used were being used out of specification.

7. Appendix 3

Current status of pasture legume symbioses

7.1 Current status of pasture legume symbioses -Research and survey results

7.1.1 Issues

Permanent pasture systems supporting beef cattle and sheep are almost totally reliant on N_2 fixation from legume symbioses to maintain their N balance and drive production. Ley and phase pastures in the wheat-sheep zone also provide N to following cereal and oilseed crops, and where they fix sufficient N to replace applications of N fertiliser they provide environmental benefits. For each tonne of fertiliser N that is replaced by legume N, between 3 and 8.5 tonne in CO_2 equivalents is saved, taking account of energy use for ammonia production, transport inefficiencies and potential N_20 emissions from N fertiliser use (Good and Beatty 2011, Jensen and Hauggaard-Neilson 2003). Legume pasture leys also provide benefits to soil structure (Puckridge and French 1983) and reduce soil borne disease in subsequent cereal crops (Kidd et al. 2002).

Without measurement, poor legume symbioses go unnoticed on all but the most N deficient of soils. There is also a perception, generated by inaccurate extension material and rudimentary assessments of nodulation, that pasture legume nitrogen fixation is satisfactory. This notion should be continuously questioned given the inconsistent relationship between nodule appearance and fixation capacity and an absence of any reliable nodulation benchmarks for comparison. Just because a plant has numerous pink nodules, it does not mean N_2 fixation is satisfactory.

This was reflected in survey outcomes of this project where interviewees were asked how they would rate the effectiveness of nodulation/rhizobial function in established stands for a range of legume species. On average, respondents indicated that they believed that nodulation was moderately effective to highly effective. When asked their reasons for such a statement, most admitted that they had no evidence or that they assumed because the seed was inoculated and/or that pink nodules were present, effective nodulation had taken place. Some of those interviewed on the other hand, were aware of evidence that indicated poor rhizobial function. The most commonly nominated issue from survey respondents was to understand and be able to measure the effectiveness of rhizobial function in the field.

The general relationship between legume shoot production and amount of N fixed (around 20 kg per t DM) means that sound agronomic practices that optimise pasture production are essential before any improvements in symbiotic function are likely to be realised. Having said this, the literature provides many examples of poor symbiotic compatibility and inadequate nodulation that indicate symbiotic function is likely to be a constraint at least to the more progressive livestock producers.

The provision of effective and persistent inoculants has played a critical role in the development of all pasture legumes in Australia. The current inoculant strains are effective with a wide range of hosts and in this regard are unlikely to require replacement soon, although a watching brief is needed to ensure that new legume varieties remain adequately serviced. There are certainly opportunities to better understand the adaptive range of inoculant strains, particularly where soils continue to acidify and farming systems evolve and place additional stresses on the symbiosis. Sub-clover, annual medic and lucerne symbioses all experience symbiotic constraints at the lower pH ranges of their distribution. Further soil acidification could see profound impacts on legume performance. Failure of the annual medic strain in saline soils provides a contemporary example of where the adaptive range of the strain was not well

understood. Efforts to select acid tolerant rhizobia in the laboratory have not succeeded, reflecting the complexity of field tolerance. Future efforts to select tolerant strains would be best done directly in the field, if required. Co-inoculants may offer some opportunity to enhance legume nodulation and growth and limited testing may be worthwhile.

The value of new strains was recognised in the survey of agronomists and further development encouraged in concert with technologies to introduce new strains into existing swards or into swards where rhizobia were absent or at low levels. Survival of rhizobia in the field as influenced by acid soil conditions and cropping rotations was rated highly.

A large body of work has used greenhouse studies to determine the symbiotic capacity of rhizobia that have naturalised in many soils. These rhizobia are likely to be responsible for more than 90% of nodulation in annual self regenerating pastures. The work has repeatedly shown that for both the annual medics and clovers the rhizobia present in soils average about 50% the fixation capacity of effective inoculant strains. There are examples of very poor symbiotic associations specific to some annual legume species and in some regions. Many new species of annual clover have been commercialised and very little information is available regarding their symbiotic capacity in the field, particularly in eastern Australia.

The significance of sub-optimal symbiotic capacity to production in the different agro-ecological zones is still to be quantified in the field and should be a priority. N application approaches and inoculation studies have been under-utilised and could be used to indicate the extent of symbiotic limitations.

Future gains in symbiotic output will largely depend on management of the rhizobia that reside in soils. Poor long term persistence by inoculant strains means that where large populations of poorly effective rhizobia are present in the soil the use of inoculants will likely have minimal long term impact. It is also counter to basic ecological principles to expect a single strain to prosper in all environments. Plants with improved symbiotic potential, if they can be developed, would assist with the management of ineffective soil rhizobia. However, it needs to be recognised that pasture renovation rates are low (<5% annually) and the approach would require a medium/long term timeframe (10+ years). The approach would also only be successful with close alignment and support from pasture breeding and evaluation programs.

A wide range of herbicides are applied pasture legumes to control grass and broadleaf weeds and are sometimes also present as soil residues where pastures are used in rotation with cereals. A number of studies as well as anecdotal reports suggest there are frequent detrimental effects of the herbicides on the symbiosis and that changes to symbiotic function may still occur in plants selected for herbicide tolerance. Assessments of herbicide efficacy based on rudimentary assessments of plant damage and regrowth are likely to be overlooking significant effects on N_2-fixation. Effect of herbicides on symbiotic function was rated very highly by survey respondents.

Inoculant quality was another issue commonly raised in the survey of agronomists. There was a high level of suspicion about the effectiveness of rhizobial seed coatings. It was unclear to those who were interviewed whether this was an issue of quality control somewhere along the supply chain or if the techniques used were being used out of specification. Quality of inoculated seed has long been a concern and some work is being undertaken by the Australian Inoculant Research Group (AIRG) to better understand conditions that favour rhizobial survival on seed and subsequently inform the coating industry. Concerns have also raised about the quality of some inoculant products and while there is scope for research to improve rhizobial titre in both seed coats and inoculants, current levels of research activity in this area seem appropriate, given that there are already high quality products and practices available that provide good levels of rhizobia to the germinating plant. The approach of endorsing products that are able to meet quality standards described in a Code of Practice administered through AIRG is preferred and should be supported.

7.1.2 Impact and extent

Thirty-two percent of soils in the study by Drew and Ballard (2010) contained populations of sub-clover rhizobia that had symbiotic capacity of ≤50%, compared to the commercial inoculant strain. Extrapolation of this data across the sub-clover area indicates potential for symbiotic limitation on 5 M ha, excluding the possible compounding effects of soil acidity in VIC. & NSW (9 M ha agricultural soils below pH 4.8). There are also regional examples showing potential for very poor symbiotic outcomes (eg symbiotic capacity of arrow-leaf clover with rhizobia in Tasmanian soils often less than 40%). For the annual medics, symbiotic capacity of the soil rhizobia is often less than 50%. This level of symbiotic capacity has been estimated to meet about half the N demand of an average medic pasture (Ballard and Charman 2000). Additional effects of residual soil herbicides in medic pastures are unknown but likely to be substantial. Severe constraints to lucerne N fixation due to poor acidity tolerance of the symbiosis are applicable to at least 1 M ha.

7.1.3 Adequacy of current information

An extensive body of published literature is available covering the impacts and management of soil acidity on clover symbioses, the fate of superseded inoculant strains and the enumeration and assessment of symbiotic capacity.

A voluminous amount of information is available examining the mechanisms of acidity tolerance and yet *in-situ* evaluation remains the only reliable method of strain selection for stressful soil conditions.

With regard to soil conditions, there is relatively little information on the adaptive range of the present inoculant strains for annual clovers and annual medic in eastern Australia.

There is inadequate information on the levels of symbiotic capacity needed to eliminate N as a principle limitation to legume production in the different livestock regions.

There are no published benchmarks for acceptable nodulation in pasture species. Negligible information is available on nodulation dynamics in perennial legumes and in particular for lucerne.

There is very limited information available on the impacts of herbicides on N_2 fixation

7.1.4 Gaps in Research and Development (In order of priority)

- Work is needed to establish a relationship between the levels of symbiotic constraint (caused by ineffective rhizobia) measured in greenhouse studies and symbiotic limitations to production in the field.
- A significant knowledge gap remains with respect to the poor nodulation and unexpected responses to inoculation measured in medic pastures in the Mallee.
- An assessment is needed of herbicide impacts on legume fixation under field conditions and examining both immediate and longer term implications.
- The contribution of plant genotype to the development of effective symbiosis requires work to (i) validate reports that some plants are able to select effective rhizobia from within large populations and (ii) examine more broadly the potential to use plant genotypes to influence symbiotic outcomes and general soil ecology.
- There is no information available to indicate if the levels of symbiotic function in soils have reached equilibrium, or are continuing to decline. It is a critical piece of information needed to make decisions on the allocation of resources for symbiotic improvement.
- Lack of a rapid (molecular) test to measure rhizobial number in soil limits the scope of ecological studies that are possible.
- The large areas of acid soils and their potential to impact on a range of legume species dictates that some work should continue in the area. Strain adaptation and development of ‘practically focussed’ screening methods are areas requiring attention.

7.1.5 Emerging Issues

Further acidification of soils combined with changed cropping practices could affect legume viability in some areas.

Symbiotic capacity may not have reached equilibrium.

Many of the research groups cited in this review are no longer active in the field. Applied N_2 fixation research at Rutherglen, Wagga Wagga, Hamilton, Canberra and Melbourne has ceased or has limited capacity. The scope for further evaluation of inoculant strains and capacity to undertake rhizobial ecological studies is very limited in eastern Australia.

7.1.6 Recommendations

1. Nitrogen or inoculation treatments should be integrated into the NVT legume program to highlight N limitations to production and followed up with targeted trials and assessments of symbiotic capacity at responsive sites.

2. Impacts of changing farming systems and soil characteristics on symbiotic function need to be quantified. The effects of herbicides on legumes critical to the wheat sheep zone (eg medic) is an area of priority. Stress tests of the commercial inoculant strains should be incorporated into this work to ensure that they remain suitably adapted.

3. The contribution of plant genotype to influence symbiotic outcomes in the field should be explored with the longer term view of providing symbiotic rankings for different varieties and influencing breeding programs to value symbiotic competency. New legume varieties should be checked to ensure that they are symbiotically competent with inoculant strains and a test set rhizobia in soils. Legumes being developed for herbicide tolerance, direct introductions from outside Australia and plants developed using wide crosses should be prioritised for testing.

4. Continued soil acidification has negative implications for legume N_2 fixation in both the permanent pasture and wheat sheep zones. A better understanding of critical pH thresholds is needed for the suite legumes being grown in each region. The information should be used to direct efforts to improve acidity tolerance of the symbiosis.

5. There is little or no capacity to undertake applied rhizobial work for the sub-clover grazing lands in eastern Australia and the appointment of a Rhizobium ecologist to work alongside legume agronomists in the region should be considered.

7.1.7 Introduction and considerations relevant to all legume species

With the exception of dairy pasture systems, negligible N fertiliser is added to Australia’s pasture systems. Thus, maintaining N balance is almost entirely dependent on inputs of fixed N from pasture legume symbioses with root nodule bacteria. Contributions from non-symbiotic nitrogen fixing microbes are likely to be small by comparison (Unkovich and Baldock 2008). Nitrogen is the nutrient exported in greatest amount from both livestock and wheat-sheep enterprises. Estimates of N exports are mostly between 20 and 50 kg/ha in West Australia and much of NSW (NLAWRA, 2001) with greater amounts of 50 to 100 kg N/ha estimated for much of the livestock areas north of the Great Dividing Range through Victoria and into south eastern NSW. Pasture legumes are widely grown in these areas.

Current understanding of the symbiotic outputs from legumes is based on field surveys of their proportional dependence on N_2 fixation, N application studies, observations of nodulation, comparison of agronomic practices that favour legume production (eg liming, provision of nutrients and inoculation) and *in-vitro* studies that have enumerated and assessed the symbiotic competency of naturalised soil rhizobia. Most approaches indicate significant opportunity for symbiotic improvement, but significant gaps exist between the conclusions drawn from *in-vitro* studies and the availability of field based evidence.

Elucidating symbiotic limitations in the field is difficult on all but the most N deficient soils. Nodulation assessments provide a valuable measure in studies where comparison to control treatments is possible as is the case in studies to understand impacts of soil acidity on symbiotic capacity (Evans et al. 1990, Richardson et al. 1988a, Coventry et al. 1987) and inoculation formulation studies (Denton et al. 2009, Roughley et al. 1993). However, except in the most extreme cases of nodulation failure or complete absence of nodule pigmentation, the measurement of nodulation has limited diagnostic value where comparisons are made across sites, due to influences of soil nitrate and differences in the effectiveness of rhizobia. The general perception that symbiotic constraints are absent where pink nodules are present is a fallacy, and simply fails to recognise that the effectiveness of symbioses can range widely regardless of nodule appearance (see Table 3: Drew and Ballard 2010). Estimates of the percentage of herbage nitrogen derived from atmospheric nitrogen fixation (%Ndfa) are also frequently misinterpreted. These data do not directly provide a measure of symbiotic capacity (although they are often used in that way) because when legumes are grown on soils deficient in available nitrogen or where soil nitrogen is quickly depleted, Ndfa will approach 100% regardless of symbiotic capacity. Conversely, low %Ndfa values may result where soil nitrate levels are high or plant growth is poor and therefore demand for fixed N low. Only where both %Ndfa and herbage %N values are low (eg 5 annual clover species with %Ndfa <42 and herbage %N<2.5 in the study by Dear et al. (2003) do these data sets point to a potential symbiotic constraint.

For all the pasture legumes presently used in Australia, the impact of inoculation practice has been profound and will continue to be important on the large areas of acid soils in cropping systems and in areas where other stresses such as salinity occur.

Where soil type is more favourable to the survival of rhizobia, large populations inevitably develop and increase in diversity, but are often less effective at nitrogen fixation. Increases in fixed N output from legumes growing on these soils will most likely arise from management practices that promote good legume content in the pasture in concert with the management of the populations of rhizobia that have developed in those soils.

7.2 Annual clovers

7.2.1 Current status of rhizobia, N_2 fixation and N contribution

Australia lacked *Trifolium* species in its indigenous flora and so all clovers and presumably their root nodule bacteria have been introduced since European settlement. It was recognised early during agricultural development that inoculation was critical to clover establishment and there were even instances where the provided inoculant strains lacked competence to colonise some WA soils (Chatel and Parker 1973) resulting in what was coined second year clover mortality syndrome.

After more than 100 years of clover cultivation (Hill 1936) the situation is now very different and the rhizobia that nodulate annual clovers are widely distributed in Australian soils. They are commonly measured at more than 1000 cells per gram in the top 10 cm of soil (Drew and Ballard 2010, Howieson and Ballard 2004, Denton et al. 2000, Quigley et al. 1997, Brockwell et al. 1982, Brockwell et al. 1975) and their distribution almost certainly extends beyond the areas where the most commonly sown species (sub-clover and balansa clover) have clover been grown, because the spread of the rhizobia has been aided by the proliferation of naturalised clover species (incl. cluster, woolly, hares-foot and hop clovers) into many environments. With the exception of gland clover, the sown species of annual clover generally nodulate with the rhizobia that have become naturalised in agricultural soils (Drew et al. 2011). The rhizobia that nodulate clovers are now probably only absent in reasonably undisturbed (natural) landscapes or occur at numbers below what is need for prompt nodulation in very acidic soils (pH<4.5) where their distribution may also be restricted to less hostile niches in the soil (Richardson and Simpson 1988).

The symbiotic capacity of clover nodulating rhizobia in soils has been the subject of many studies. Early work attempted to quantify the effectiveness of individual isolates (Brockwell et al. 1982, Gibson et al 1975) whist more recent studies have relied on the whole soil inoculation technique (Quigley et al. 1997, Brockwell et al 1988, Bonish 1979) to provide an integrated assessment of the symbiotic capacity of soil communities. What both approaches clearly show is that individual strains and the communities of rhizobia that reside in soils generally have less, often much less, nitrogen fixation capacity than commercial inoculant strains. For sub-clover recent estimates indicate that average symbiotic capacity of the soil rhizobia is about 50% compared to inoculant strain WSM1325 (Drew et al. 2011) with 32% of soil communities classed as poorly effective. The level of symbiotic capacity can be modified by legume host with the effectiveness of soil rhizobia with alternative clovers generally similar or less than for sub clover (Drew et al. 2011, Brockwell et al. 2008, Ballard 2002, Denton et al. 2000). A specific example of such an interaction is provided by Drew et al (2011) where the mean effectiveness of symbioses formed by arrow-leaf clover (42%) was poor when compared to sub-clover nodulated by the rhizobia that reside in Tasmanian soils. Detailed interactions between legume genotypes and strain of rhizobia have been extensively studied, initially prompted by nodulation failures between Woogenellup sub-clover and inoculant strain TA1 (Gibson 1968) and more recently focussed on the various clover species that are now available to growers (various clovers-Drew et al. 2011, rose clover-Brockwell et al. 2008, various clovers-Denton et al. 2000, purple clover-Yates et al., 2008, various clovers-Howieson et al. 2005). This body of work on the effectiveness of clover rhizobia overwhelmingly shows that the symbiotic capacity of rhizobia in soils falls well short of the inoculant strains.

Various field surveys provide estimates of the percentage of herbage nitrogen derived from atmospheric nitrogen fixation (%Ndfa) for subterranean and other annual clover species using the natural abundance ^{15}N method (Denton et al. 2011, Dear et al. 2003, Peoples et al. 1998, Quigley et al. 1997, Bolger et al. 1995, Sanford et al. 1994). The value of these data sets has largely been to establish relationships between legume dry matter production and contribution of total fixed nitrogen, providing comparative data for different legume genotypes and quantifying variation between sampling locations. Reviews of such data by Peoples and Baldock (2001) and recently by Unkovich et al. (2010) indicate that in general, subterranean clover plants derive >65% of shoot N from fixation, although Sanford et al. (1994) reported that 29% of WA sub-clover pastures fell below this benchmark. The only discrimination between sub-clover genotypes is made by Dear et al. (2003).

Where %Ndfa data is combined with measures of herbage production, a relationship between amount of nitrogen fixed per ha and legume production is apparent. For annual clovers (mostly subterranean clover) about 20 kg of fixed N is measured per tonne of shoot dry matter (DM) production Unkovich et al. (2010). While this relationship has been heavily promoted and is generally accepted, variation about the mean (20 ± 10kg/ha fixed N per tonne; refer upper and lower data boundaries, Fig. 1, Peoples and Baldock 2001) indicate substantial opportunity to increase average nitrogen contributions per unit of dry matter if the reasons for the variation are able to be elucidated. Further, to account for root contributions which are unable to be reliably measured, Unkovich et al. (2010) suggests a conservative multiplier of 1.5 is used, which has the effect of increasing the difference of the upper and lower estimates of fixed N per tonne of shoot DM production. While issues of auto-correlation tend to over-state the strength of the relationship between DM production and total fixed N production, the association does nonetheless highlight the importance of legume production to maximising inputs of fixed N. In this regard, the average contributions by annual clovers of 65 kg/ha of fixed N (Unkovich et al. 2010) would just meet N balance requirements in many livestock regions, and falls far short of the 150 kg/ha reported for some annual clover pastures.

7.2.2 Issues

7.2.2.1 Efficacy of inoculants and dealing with ineffective soil rhizobia

Significant effort has been directed at the selection of elite inoculant strains for application at pasture renovation and to optimise the symbiotic potential of new clover species especially when sown in areas where clovers may not have previously grown and on acid soils. The current strain for annual clovers (WSM1325) is highly effective across a broad range of clover species, many of which were less well serviced by former long term inoculant strain WU95 (Drew et al. 2011, Reeve et al. 2010b, Howieson et al. 2005).

Inoculant strains of rhizobia when carefully applied can achieve high levels of nodule occupancy even where large populations of soil rhizobia occur (Ireland and Vincent 1968) but the occupancy of nodules by inoculant strains generally declines from season to season (Slattery and Coventry 1999, Brockwell *et al.* 1982). With specific reference to WU95, the annual clover inoculant from 1974-1999 (Bullard et al. 2005) the strain was found to occupy less than 18% of sub-clover nodules just one year prior after application at pasture renovation and was not able to be detected a year later (Slattery and Coventry 1993). Similarly, Sitepu (2001) found that WU95 was rarely, if ever, identified as an occupant of *T. michelianum* (cv. Paradana) nodules formed after inoculation with soils collected from clover pastures. We are not aware of any ecological studies on the fate of more recent clover inoculant strains (WSM409 and presently WSM1325) in eastern Australia, but it seems contrary to ecological principles that a single inoculant strain should be able to survive and compete across the broad range of environments where clovers are sown. It is suffice to say that while there are specific instances of inoculants strains maintaining high nodule occupancy in the field (e.g. Gibson et al. 1976) by and large the benefits of effective strains introduced through inoculation are temporary and possibly insignificant where the pasture phase extends past a few years.

Opportunities for managing soil rhizobia are more likely to emerge from a better understanding of how different plant genotypes interact with communities to sometimes increase nodule mass or influence which rhizobia form nodules. Once again *in-vitro* studies provide sometimes dramatic evidence of the potential for plants to influence symbiotic outcomes (Drew et al 2011, Drew and Ballard 2010, Yates et al. 2008) but these possibilities have not yet been demonstrated to any significant extent in the field in Australia. It is an area worthy of further work.

Momentum with respect to the development of new inoculants is shifting towards the evaluation of non-rhizobial inoculants or co-inoculants that may provide benefits to the legume in their own right, or interact synergistically with the legume and/or rhizobia to improve nodulation or N fixation. While we are not aware of published data demonstrating improved pasture legume performance under Australian field conditions, opportunities that arise in the area may be worth some level of assessment.

7.2.2.2 Maintenance of symbioses on acidic soils

Many of the studies aiming to overcome symbiotic constraints in sub-clover pastures have been appropriately targeted at highly acidic soils ($pH_{Ca}<4.8$) which are extensive (conservatively estimated at 12.5 M ha, Anon 1997) in the regions where sub-clover is grown as permanent pasture and in cropping rotations. Sub clover is able to grow on these soils partly because of its relatively good tolerance of acidity compared to other legumes (Evans et al. 1990), but symbiotic capacity is often reduced. Effects of soil acidity on the clover symbiosis has been extensively studied in the field with impacts on rhizobial survival in soil and nodulation well described (Slattery et al. 2001, Slattery and Coventry 1999, Evans et al 1988, Richardson and Simpson 1988, Richardson et al. 1988a, Coventry et al. 1987, 1985).

The impacts of acidity on clover symbioses can become apparent at pasture renovation, especially where clover has been absent for some time, for example after an intensive cropping phase (Coventry et al. 1985). Field studies are supported by many mechanistic studies and reviews that collective show that many factors limit the formation of clover symbioses under acidity stress (Watkin et al. 2003, Dilworth et al. 2001, Chen et al. 1993, Richardson et al. 1988b).

While the application of lime provides an obvious way of ameliorating the effects of soil acidity, projections of further soil acidification (Anon 1997) and localised effects of lime applications where it is not incorporated (Richardson and Simpson 1988) have seen a body of work undertaken to select strains of rhizobia with acidity tolerance. Successful outcomes still remain tied to *in-situ* field selection (Watkin et al. 2000) despite numerous attempts at *in-vitro* selection on acidified agar (Gemell and Roughley 1993 Richardson and Simpson 1989, Slattery and Coventry 1995). The failure of *in-vitro* approaches thus far reflects the complexity of acidity tolerance and significant knowledge gaps in the area despite the substantial body of work completed. There is also a tendency for strains isolated from very acid soils to have reduced symbiotic effectiveness.

The competence of current inoculant strain WSM1325 on acid soils has been shown in WA but limited testing has been completed in south-eastern Australia, particularly in combination with the ‘alternative’ annual clovers that are now available and likely to be used on some very acidic soils.

7.2.2.3 Effects of pasture management

Pasture management practices that favour good clover content and remove primary limitations to growth should be first considered in any attempts to improve contributions of N from fixation. Such practices are well known and are routinely undertaken. They include the provision of adequate nutrition, particularly P (Peoples et al. 1998) and those trace elements (e.g. Mo, Co and B) needed and often specific to nodule development and function (see review by O’Hara et al. 1988). Lime application in sub-clover pastures is often recommended to a level where Al damage to roots is minimised (J. Shovelton pers. com.) and in this scenario where $pH_{(water)}$ is increased above 5.0 impacts on the symbiosis will be much reduced, even though effects on the rhizobia are still measureable (Fig. 1; Slattery and Coventry 2001). Where severe subsoil acidity is present (Scott et al. 2000) or where liming is not practiced and soils continue to acidify, symbiotic failure may emerge as a major issue in the longer term. Here management to reduce the rate of acidification is needed but should probably focus on the development and inclusion of perennial pasture plants into these areas (Dear et al. 2009). This approach should not exclude the selection of inoculant rhizobia with acidity tolerance for these areas.

Nodulation and N_2 fixation are sensitive to soil nitrate (Carroll and Mathews 1990, Herridge et al. 1984). In long term pastures, as soil nitrate increases, legumes are either replaced by grasses and nitrogen loving herbs, or where clovers persist in the pasture they fix less N. Options to manage this are limited in permanent pastures other than by increasing the content of perennial grasses to reduce available soil nitrate and using grazing or other practices that encourage clover persistence. Unkovich and Pate (1998) reported variation in nitrate tolerance between different populations of rhizobia under sub-clover pastures, but how this can be practically used is unclear. In ley-farming systems there is more opportunity to manage soil nitrate level. In these systems length of the legume and cropping phases affects available soil N levels (reviewed by Young, 1988) and therefore provides opportunity to manage them. Brockwell et al. (1995) pragmatically suggest avoiding planting legumes into soils containing significant amounts of available N. Avoiding long fallows, minimising tillage and incorporating C rich residues are strategies that can be used to further reduce N levels at the beginning of the pasture phase. Nitrate tolerant plants with hyper and super-nodulating traits (Bhatia et al. 2001, Carroll et al. 1985) have been developed for many species of crop legumes. Similar work is plausible for the pasture legumes, but efforts would need to be closely aligned with breeding programs in order to deliver a commercial outcome.

The use of herbicides to encourage legume dominance is cited by Peoples et al. (1998) as an important tool for improving N fixation in sub-clover pastures and is an accepted practice in ley pasture systems. The impact of many herbicides on N fixation remains unknown and is an area requiring investigation.

There are surprisingly few studies that examine grazing on the symbiosis. A study of sub-clover by Sanford et al. (1995) showed that a higher amount of fixed N was recovered under grazing, largely due to increased overall pasture productivity, even though heavy defoliation in winter temporarily reduced fixation.

7.2.2.4 Impact and extent

Annual clovers are grown extensively in both the sheep-wheat and perennial pasture zones, occurring on more than 20 M ha of grazing lands.

It has been recognised for a long time that sub-clover production levels fall well short of water limited potential (Black 1964) and studies in south-east SA (520mm annual rainfall) have shown there is potential for large increases in sub-clover production if N supply can be increased (Cocks 1980). Unfortunately, that study failed to elucidate the limitations to N supply, although others have measured responses to inoculation and suggest that poorly effective soil rhizobia play a significant role (Leo Hamilton pers. com.). This is plausible given the overwhelming evidence showing that the rhizobia in soils are often only 50% as effective as commercial inoculant strains. Even so, work is needed to define the level of symbiotic capacity required under field conditions. In situations where legume growth rate or total production are not high, where soil N mineralisation supplements N needs, or the requirement of N export is low, moderately effective symbioses may be adequate to meet N demand of the production system. A better understanding will ensure efforts to improve the symbiosis are targeted into responsive areas.

Sub clover is grown extensively on soils which are acidic and continuing to acidify. Coventry et al. (1987) has demonstrated that the symbiosis is a key limitation to sub-clover nodulation and production on these soils. In the absence of liming, large areas are at risk of decreased production and little is known of the adaptive range of the current inoculant strain for clover in eastern Australia.

7.2.2.5 Adequacy of current information

Improvement of clover symbioses is limited by knowledge gaps pertaining to:

- Extent to which ineffective symbioses limit field production in different agro-ecological zones.
- Lack of understanding of the influence of plant genotype on the development of effective symbiosis.
- Poor definition of the relationship between clover nodulation and production.
- Limited data on the adaptive range of current inoculant strain on acid soils. Insufficient knowledge to enable reliable strain selection for acid soils.

7.3 White clover

7.3.1 Current status of rhizobia, N_2 fixation and N contribution

White clover (*Trifolium repens*) dominates sowings of perennial clovers and has used inoculant strain TA1 since 1961 (Bullard et al. 2005). It is mainly grown in the high rainfall districts in NSW, VIC and Tasmania.

In general terms, perennial clovers demonstrate more specificity for effective symbiosis than their annual counterparts (Howieson et al. 2005). White clover forms effective symbioses with inoculant strain TA1. However it commonly forms sub-optimal symbioses with the inoculant strains that have been used for annual clovers (Ballard unpub. data) the implication being that there is potential for ineffective symbiotic associations where the distribution of the annual and perennial clover species overlap.

The most recent surveys of white clover pastures in south western Victoria (Riffkin et al. 1999a, 1999b) indicate %Ndfa values of around 65%. Number of rhizobia generally exceeded 10,000 per g soil and they were on average about 50% as effective as the best treatment. Amounts of N fixed were very low, often less than 20 kg/ha but this was largely due to low legume content in the pasture. Quigley et al. (1997) also sampled 3 pastures containing white clover in the same region and went on to measure effectiveness of the rhizobia on sub-clover. Effectiveness of the rhizobia ranged from 47 to 83%. A small study by Charman (unpub. data) showed the rhizobia in 3 soils were shown to be less than 50% effective.

7.3.2 Issues

While there is evidence to show the rhizobia in white clover pastures are sub-optimal for fixation and within similar ranges for sub-clover, the factor most likely to influence overall nitrogen contribution is legume content. The application of nitrogen fertilisers to perennial pastures to drive grass production is increasing, particularly under irrigation. Comprehensive reviews on the subject are given by and Eckard (1998) and Mundy (1999) and demonstrate where N fertiliser is repeatedly applied, both N_2 fixation and clover content are diminished. There is some room to manipulate levels of N_2 fixation in these systems through the strategic application of N fertiliser (Mundy 1999) and minimisation of drying and water-logging episodes under flood irrigation regimes. While there are examples of plant genotype affecting fixation potential (Chapman and Caradus 1997, Mytton and Rys 1985) the management of N inputs should precede any attempts to improve the symbiosis *per se*.

With regard to long term inoculant strain TA1, there are concerns about both its survival on seed (Gemell et al. 2005) and colonisation in acid soils (Watkin et al. 2005). Progress against both criteria seems plausible, but would only be of value where the application of nitrogenous fertilisers is well managed.

7.4 Annual medics and symbiotically allied genera

7.4.1 Current status of rhizobia, N_2 fixation and N contribution

The annual medics and several allied genera that form nodules with the same rhizobia are considered in this section. A number of medic species exploited commercially show different preferences with regard to the strains of rhizobia that they form effective symbioses with (Howieson et al. 2000, Ballard and Charman 2000, Brockwell et al. 1988, Brockwell and Hely 1966). The genera *Trigonella* (Howie et al. 2001) and *Melilotus* (Nichols et al. 2010) which also have substantial potential for development as pasture species also have a tendency to need specific rhizobia for effective symbiosis. It is because of this specificity that two commercial inoculant strains (WSM1115 and RRI128) are provided for the different species of annual medic and a separate strain (SU277) provided for Fenugreek (*Trigonella foenum graecum*). In a practical sense this specificity predisposes legumes within this complex to form suboptimal symbioses where the source of rhizobia is uncontrolled. It is ironic that a basic failure to recognise the importance of symbiotic specificity within the *Medicago* has seen the major global investment in the sequencing *M. truncatula* (A17) and a *Sinorhizobium* as the model legume system inadvertently choose an incompatible micro-symbiont for the work (Terpolilli, 2008). The practical implication of such specificity is that a watching brief is needed to ensure that new cultivars, particularly where hybridisation or mutation has been used, remain compatible with inoculant strains and the rhizobia that occupy many soils.

Soils of neutral or alkaline pH that have some history of medic presence usually contain large populations (>1000 per g soil) of naturalised rhizobia (Howieson and Ballard 2004, Brockwell 2001, Brockwell et al. 1991). Studies of these rhizobia have been somewhat contradictory with (Brockwell 2001) suggesting the rhizobia are effective while others (Ballard and Charman 2000) have concluded they are often sub-optimal. The cause of these contradictions appears to be varying effectiveness of control treatments used by the different research groups and it is now generally accepted that annual medics are prone to the formation of sub-optimal symbioses with soil rhizobia. Studies by Ballard and Charman (2000) and Charman and Ballard (2004) that benchmarked performance of the soil rhizobia against highly effective strains indicate that average efficiency of soil rhizobia is less than 50%. Strong Interactions with plant genotype occur, with burr medic notable for developing particularly poor symbioses (Charman and Ballard 2004). Some species (eg *Medicago rigiduloides*) only form ineffective symbioses and great care needs to be taken to ensure that they are not used in the development of legume material for Australian farming systems.

The extent to which poor symbiotic capacity limits legume production in the field is still to be quantified, but the frequency of very poor symbiotic associations measured indicates it is likely to be substantial, at least where medic production has the potential to be reasonable.

Estimates of the percentage of herbage nitrogen derived from atmospheric nitrogen fixation (%Ndfa) for the annual medics was summarised by Peoples and Baldock (2001) and for the Australian data ranged from 18-99%, with corresponding large variation in the amount of N fixed (2-220 kg/ha.year). Dear et al. (2003) provides a small amount of additional data indicating fixation rates between 50-70%. These values indicate there is potential to improve the proportion of N derived form the atmosphere, but do not indicate poor symbiotic function *per se*.

7.4.2 Issues

7.4.2.1 Soil adaptation

The sensitivity of medic rhizobia to soil acidity is well known (Brockwell et al. 1991) and has been the subject of a large work program to select acid tolerant inoculant strains (Howieson and Ewing 1986), particularly with regard to the AM inoculant (WSM1115) for application to the more acid tolerant species of medic (eg *polymorpha*, *murex* and *spherocarpus*). It has no doubt resulted in better medic nodulation on acidic soils (Denton et al 2007, Barclay et al. 1994, Howieson and Ewing 1989) and will continue to provide value where inoculation plays an important role in the re-establishment of legume pastures (eg Bowman et al. 1998). In areas where soils continue to acidify (measures of pH 6.5_{water} now common for Mallee sands) further work will be needed to ensure the nodulation of acid sensitive medic species is not compromised. Work to understand the mechanisms that confer acidity tolerance in medic rhizobia has been extensive (e.g. Reeve et al. 2006, see review by Dilworth et al. 2001) and made progress in identifying some of the prerequisite genes for acidity tolerance and shows that tolerance is complex and likely to be under the control of at least 50 genes. Recent sequencing of acid tolerant strain WSM419 expected to provide further insights (Reeve et al. 2010a) in the longer term. Nonetheless, large knowledge gaps remain, and as a result field selection remains the only reliable approach for the selection of acid tolerant strains.

In broad terms, numbers of medic rhizobia are lower in WA soils (Ballard unpub. data). This is probably related to the effects of combined stresses of acidity and low clay content (Howieson and Ballard 2004).

Recently the commercial AM inoculant strain (WSM1115) has been shown to have poor persistence in very saline soils (Bonython et al. 2011). An absence of adapted host plants (medics and allied species) has also meant that any naturally adapted rhizobia have lacked the means to establish in these regions. The development of Messina (*Melilotus siculus*) for these soils has been undertaken in concert with rhizobial selection work and several adapted strains have been identified. If Messina were to develop into a significant legume species, further assessment of the adaptive boundaries of the symbiosis may be warranted.

7.4.2.2 Changing farming systems

Medics are widely used in the cereal livestock zone as a component of the farming rotation. The extent to which symbiotic efficiency is being affected by changes in cropping frequency, increased use of broadleaf and grass selective herbicides or the residual fertiliser N from the cereal phase is not well understood. There are however consistent reports of poor medic nodulation and this has recently been verified in evaluation trials at different locations in the SA Mallee (Ballard unpub. data 2011). The extent of this nodulation failure was unexpected and the reasons remain to be elucidated.

Certainly there is evidence to show that herbicide residues in soils affect nodulation. Impacts of broad-leaf herbicides, particularly those in the Group B class have been shown to profoundly affect root development of annual medics (Farquharson 2009) and have been implicated in the poor persistence of lucerne (Koopman et al. 1995). Other herbicides have similarly been shown to have impacts on legume N_2 fixation in cropping systems (Drew et al. 2007) and observations of altered nodule appearance after the application of grass selective herbicides have been reported, but there has not been any systematic study of their impact on pasture nodulation or N_2 fixation. Most assessments of herbicides in pastures simply rely on rudimentary assessments of herbage damage and recovery (see Sandral et al. 1997). The development and commercial release of Angel strand medic (tolerant to a number of Group B herbicides) has lessened the impact of Group B herbicides (Howie and Bell 2005) but even here there remain some concerns with respect to altered symbiotic function (Farquharson 2009). Transfer of the tolerance trait to other medic species will likely see increased use of tolerant varieties and so it would be prudent to ensure that adequate symbiotic function is maintained. In more general terms, the impacts of herbicides on both nodulation and symbiotic function should be a high priority.

The nodulation of annual medics is sensitive to soil nitrate, with Ewing and Robson (1990) reporting reduced nodulation at very low levels of nitrate in solution culture with barrel medic being most sensitive. Butler (1988) similarly reported 50% reductions in fixation for strand medic at relatively low levels of applied nitrate (20 mg nitrate/kg soil) at germination, but showed that effects could be mediated in the presence of ryegrass competing for the nitrate. Paradoxically, the grass ‘freeing’ of medic pastures to optimise production and the disease break effect is likely to reduce N_2 fixation per plant, although it is generally accepted that maximising legume biomass is more important to overall nitrogen contribution. Sensitivity of the medic symbiosis to soil N may become more important if application rates of fertiliser N continue to increase (Angus, 2001) especially if residual N is carried into the pasture phase. In the wetter margins of the wheat-sheep zone, application rates in crop can exceed 50 kg/ha (source GRDC agro-ecological zone statistics). There has also been a recent trend to add N fertiliser at pasture sowing because it is 'cheap' and may assist the early vigour of non-legumes, but seems contrary to the encouragement of N_2 fixation. Soil N should be able to be managed using appropriate rotational sequences to minimise carry over into the pasture phase, so any work in this area should first be to establish that in well managed systems soil nitrate level is linked to reduced nodulation or N_2 fixation. If nitrate level is not able to be managed, the selection of plant genotypes with reduced nitrate sensitivity could be used to lessen the impact. Collections of nodulation and fixation mutants that include hyper-nodulating NTS lines (Schnabel et al. 2010) could be used to better understand the potential to use plant selection, if access to the collections were possible. Such a use would extract value from material that is mostly used in esoteric studies.

Studies investigating the syndrome of medic decline in the 1990's (Brockwell 2001, Slattery unpub. data - GRDC project UA345, 1998-2002) concluded that the large numbers of medic rhizobia measured in the mostly neutral to alkaline soils in the study areas were numerically sufficient for the prompt nodulation of medics. This seemed reasonable and large populations have since been measured by Ballard and Charman (2000) and Charman and Ballard (1994). However none of the studies proceeded to examine response to inoculation in the field. Unexpected and large responses to inoculation in recent field work (Ballard unpub. data, 2011) indicate the soil rhizobia may not be providing sufficient inoculum potential which is at odds with earlier findings. It may therefore have been premature to dismiss rhizobia as a contributing factor to medic decline. Inoculation responses in the field may be the result of the proximity of the applied rhizobia to the germinating root or associated with signalling between the plant and the provided inoculant strains (Howieson et al. 1992). A knowledge gap exists regarding the reasons for the inoculation responses that require clarification.

7.5 Dealing with poorly effective soil rhizobia

For the reasons outlined for annual clovers, the use of inoculants to overcome the soil rhizobia is unlikely to extend beyond a few years after pasture renovation. Plant selection provides some scope for improvement (Nair et al. 2004) and for the strand medics a cohort of symbiotically promiscuous strand medic material has been produced, but it is still to be examined to any significant extent in the field. There appears less potential to use this approach in burr medic (Charman and Ballard, 2004).

7.5.1 Impact and extent

Annual medics are grown extensively in the sheep-wheat zone, particularly where regeneration after a cropping phase is desirable. Areas are vast and estimated to exceed 20M ha (Hill and Donald 1988). Barrel and burr medic are most widely distributed, although species such as strand medic play critically important regional roles on the coarse textured Mallee sands in SA and VIC.

The impact of poor symbioses is substantial. For burr medic, soil rhizobia are about 40% effective (Charman and Ballard 2004), poor in about 50% of soils tested and equally distributed in soils from NSW, VIC and SA. For strand medic, the soil rhizobia are reported to be 49% effective in a study of SA soils (Ballard and Charman 2000). Relatively little data is available for WA because low rhizobia number is more often the primary constraint to symbiosis. Because poor rates of pasture renovation are a generic problem in the lower input regions where medics are grown, using cultivars developed to be compatible with the soil rhizobia would require a long term commitment. Having said this, the extent of poor symbioses measured means some work is warranted.

Practice change to ensure prompt and abundant nodulation has the potential to deliver widespread benefits in a relatively short time frame. First the factors leading to poor nodulation need to be elucidated and appropriate recommendations made where a clear link to grower practice (eg herbicide, fertiliser applications and crop sequence) is established.

7.5.2 Adequacy of current information

Improvement of medic symbioses is limited by knowledge gaps pertaining to:

- Reasons for poor nodulation and subsequent responses to inoculation in Mallee soils
- Effects of herbicides on symbiotic function
- Extent to which ineffective symbioses limit field production in different agro-ecological zones
- A lack of reliable *in-vitro* screening methods is available for strain selection because of inadequate understanding of the mechanisms that provide strains with adaptive advantage in soils.

7.6 Lucerne

7.6.1 Current status of rhizobia, N_2 fixation and N contribution

Lucerne forms symbiotic associations with the same rhizobia that nodulate annual medics, but generally demonstrates far less symbiotic specificity for effective symbiosis (Bowman et al. 1998, Brockwell et al. 1995). Another example of reduced specificity is provided in a study of the rhizobia in 50 soils from south-east SA where the lucerne varieties Trifecta and Sceptre formed effective symbioses, almost without exception (Ballard et al. 2003).

As described in the annual medic section above, soils of neutral or alkaline pH that have some history of medic or lucerne presence usually contain large populations (>1000 per g soil) of naturalised rhizobia. Significant numbers of rhizobia have also been measured at depth in the soil profile (30-60 cm) under lucerne pastures (Evans et al. 2005a, Brockwell et al. 1995) where has been suggested they may be important to maintaining N_2 fixation when conditions at the soil surface (eg during dry summers) are not conducive to nodule formation or N_2 fixation. Information on the dynamics of lucerne nodulation is sparse. Gault et al. (1995) reports the appearance of 'renewed' nodules on lucerne roots after grazing and we have made similar observations upon the resumption of lucerne growth post break of season (Ballard pers. com.). The implication here is that a source of persistent rhizobia is almost certainly needed in the soil to enable these re-nodulation events.

An estimate (67%) of the percentage of herbage nitrogen derived from atmospheric nitrogen fixation (%Ndfa) for 39 lucerne pastures is provided in the review by Peoples and Baldock (2001). Gault et al. 1995 reported rates between 65 and 95% for established lucerne under irrigation, with total amount of fixed N exceeding 200 kg/ha/year. Bowman et al. (2004) has suggested that N_2 fixation is limited at low temperatures and this may limit the potential for its use in particularly cold environments.

7.6.2 Issues

7.6.2.1 Soil adaptation

The most important constraint to the lucerne symbiosis is its sensitivity to acidity. Early work of Munn's (1968) clearly showed the presence of an acid sensitive step immediately before root hair curling, early in the nodulation process. After root hair curling and development of the infection thread, symbiosis and plant growth is able to proceed relatively normally, even at vey low pH (<4.5). Sensitivity of medic rhizobia in terms of their survival in acidic soils is also well known (Brockwell et al. 1991) and evidence is provided by Evans et al. (2005b) demonstrating the advantages of some strains to lucerne growth and persistence in acidic soils in WA. Further work using solution culture methods to select strains able to nodulate at pH 4.8 is in process (Charman et al. 2008), with selected strains showing better persistence and nodulation in the field (Ballard et al. 2008, unpub. data).

Selection of more acid tolerant strains is being undertaken in parallel with the selection of acid tolerant plant germplasm. Relative contributions of the plant and rhizobia to improved symbiosis in the field are still to be determined as are the adaptive boundaries of the improved symbiosis.

7.6.2.2 Herbicide impacts

Lucerne pastures are often maintained for 2-3 years on cropping systems, but this commonly extends to 10 years in permanent pasture systems (see Table1; Ballard et al. 2003). Herbicide use is commonplace for weed management, with a mixture comprising spray seed and diuron often used for the control of annual grasses and broad leaf weeds. Glyphosate tolerant lucernes are also likely to enter the Australian market in the next decade (A. Humphries, pers. com.). Studies by (Eberbach and Douglas 1989, 1991) have shown detrimental effects of various components of the above herbicides on the symbiosis in subterranean clover. Chlorsulforon residues have also been implicated in the poor persistence of lucerne (Koopman et al 1995). Some investigation of herbicide impacts on lucerne should be a priority. The ability of lucerne to access N at depth in the soil means that impediments to the symbiosis after herbicide application can be easily overlooked.

7.6.2.3 Impact and extent

Lucerne is presently grown on approximately 3.5 M ha and is projected to increase to 7 M ha. Most of this expansion will occur in northern Victoria and southern NSW (A. Humphries pers. com.) where up to 50% of soils are classed as moderately to very acid (between pH 5.5 to 4.3 (calcium), Anon 1997) with a high proportion occurring in the areas foreshadowed for lucerne expansion (Robertson 2006) The area of lucerne in WA is presently less than 100,000 ha and further perennial legume expansion in that state is more likely to rely on Tedera because of its superior drought tolerance (C. Revell pers. com.). Based on our current understanding of the pH constraints on the lucerne symbiosis we estimate that an improvement in acidity tolerance of 0.25 of a pH unit would expand the area of land for reliable lucerne establishment by at least 1 M ha.

Herbicides are routinely applied to lucerne and are also likely to persist as soil residues particularly in the lower rainfall cereal livestock zone. Since lucerne is able to access N at depth in the soil, perturbations to the symbiosis after herbicide application are likely being overlooked. No systematic assessment of their impact on symbiotic function is available and this is seen as a priority.

7.6.2.4 Adequacy of current information

Improvement of lucerne symbioses is limited by knowledge gaps pertaining to:

- The lack of correlation between in-vitro strain selection at low pH and performance of the strains in the field. Relative contributions of the plant and rhizobia to acidity tolerance in the field.
- Understanding the nodulation dynamics of perennial legumes. Included here is the lack of tools to efficiently examine rhizobial population changes.
  - Quantitative data on the effects of herbicides on symbiotic function

7.7 Other legume species (In order of current sown area)

7.7.1 Serradella (Ornithopus species)

There are several species of serradella with the yellow (*O. compressus*) and pink (*O. sativus*) species most commonly sown, probably on more than 1 M ha in total; mainly in WA and NSW. It is best grown on infertile free draining neutral or acidic sands. It is nodulated by the same rhizobia as lupin (*Bradyrhizobium* spp. *lupinus*). Inoculant strain WSM471 is used in WA based on data showing superior effectiveness with pink serradella and improved soil colonisation (Ballard 1996) whilst WU425 has performed better at some sites in NSW (Hartley and Gemell, 2004) and is used in that state.

Once established the rhizobia are persistent in the soil for many years with genetic studies by Stepkowski et al. (2005) indicating the strains in WA soils are descendants of strains originating from Europe. Generally the strains in soils are effective for N_2 fixation (McInnes 2002) and produce abundant nodulation where soil pH is less than 7.5.

There are no immediate issues requiring attention.

7.7.2 Lotus (Lotus species)

There are many species of *Lotus* and they include both perennial and annual forms. *Lotus corniculatus* and *Lotus pedunculatus* are grown in Australia but are largely been restricted to the high rainfall (>600mm) regions and remain relatively minor species. Species within the genera are highly specific in their rhizobial requirements for effective symbiosis (Howieson et al. 2011, Gault et al. 1994). Recent efforts to further exploit the genera have seen the extensive evaluation and selection of birds-foot lotus (*L. ornithopodioides*) for possible commercialisation. An annual species, it is likely to have wider adaptation than those species previously used. An extensive work program to select suitable rhizobia has also been completed (Howieson et al. 2011) and effective rhizobia for the species are available. The selected rhizobia have good levels of acidity tolerance (Ballard unpub. data). There are some rhizobia naturalised in soils that are able to nodulate *Lotus*, but the host plants are so infrequent in broad-acre farming systems that inoculation remains a pre-requisite for successful nodulation. The rhizobia for *Lotus* (at least those that nodulate *L. corniculatus*) are predisposed to horizontal gene transfer via the exchange of a symbiosis island with other soil bacteria (Sullivan and Ronson 1998). This results in the rapid development of ineffective rhizobial communities in the soil and this should be monitored if birds-foot lotus gains significant momentum as a commercial species.

There are no immediate issues requiring attention.

7.7.3 Biserrula (Biserrula pelecinus)

Biserrula is an introduction from northern Africa and the first cultivar was released in Australia in 1997. It is now sown on significant areas in WA and the plant is also showing significant potential in parts of NSW (B. Hackney pers. com.). The plant forms a highly specific symbiosis with *Mesorhizobium ciceri* bv. *biserrulae* (Nandasena et al. 2007b).

Of most interest has been the rapid *in situ* evolution of less effective nodulating strains for legume in WA soils (Nandasena et al. 2007a) as a result of the transfer of a symbiotic island to other soil bacteria, similar to that described by Sullivan and Ronson (1998) for *Lotus*.

The initial and type inoculant strain (WSM1271) was replaced by WSM1497 in 1998. The introduction of this legume into new environments provides the opportunity to examine the development of ineffective symbioses through time and possibly examine the potential of different strains to mediate this process.

There are no immediate issues requiring attention

7.7.4 Sulla (Hedysarum coronarium)

Sulla is the only species used commercially used in Australia, although several others including *H. flexuosum* and *H. carnosum* have been assessed in evaluation programs. It has a specific rhizobial requirement (Kishinevsky et al. 1996, Casella et al. 1984,) and must be inoculated because the plant has not been widely grown and suitable rhizobia are generally absent from Australian soils. The species is generally recognised to prefer neutral or alkaline soils and medium to high rainfall. Rhizobial strain WSM1592 replaced CC1335 in 2007 based on improved colonisation by the latter strain in a mildly acidic field soil in SA (Ballard and Charman Unpub. data). Testing of the adaptive range of the strain is limited.

There are also reports of poor strain survival on seed, and this has the potential to significant undermine adoption of the species. Several cultivars (Wilpena, Moonbi and Flamenco) have been released in the last five years and have the potential for high herbage production (Yates et al. 2006) and are therefore reliant on adequate N_2 fixation.

Some further assessment of the adaptive range of rhizobia and their survival on seed is probably warranted to ensure the development of this legume is not unnecessarily constrained.

7.7.5 Cullen (Cullen australasicum)

Cullen is a native perennial legume being evaluated for potential use in Australia (Dear et al. 2008). Effective strains of rhizobia are available to enable evaluation but no information on the adaptation of symbiosis to soil conditions is known. There is no information available on the need to inoculate in agricultural soils. Both would need to be clarified before a commercial release.

There are no immediate issues requiring attention.

7.7.6 Tedera (Bituminaria bituminosa var. albomarginata)

Tedera is a perennial legume, introduced into Australia from the Canary Islands. It needs to be inoculated, since there are unlikely to be suitable rhizobia in Australian soils. Rhizobial strains for evaluation are available through Murdoch University. It has been mostly evaluated in WA and so the extent of adaption of both plant and rhizobia is still to be determined.

7.8 References

Angus JF (2001) Nitrogen supply and demand in Australian agriculture. *Australian Journal of Experimental Agriculture*. **41**, 277-288.

Anon (1997) Australian Natural Resources Atlas. (www.anra.gov.au/topics/soils /acidification).

Ballard RA (1996) Assessment of strains of *Bradyrhizobium* sp. (*Lupinus*) for serradellas (*Ornithopus* spp.). *Australian Journal of Experimental Agriculture* **36**, 63-70.

Ballard RA, Charman N (2000) Nodulation and growth of pasture legumes with naturalised soil rhizobia 1. Annual *Medicago* spp. *Australian Journal of Experimental Agriculture* **40**, 939-948.

Ballard R, Craig A, Charman N (2002) Nodulation and growth of pasture legumes with naturalised soil rhizobia. 2. Balansa clover (*trifolium michelianum* savi). *Journal of Experimental Agriculture* 40: 939-948.

Ballard RA, Shepherd BR, Charman N (2003). Nodulation and growth of pasture legumes with naturalised soil rhizobia. 3. Lucerne (*Medicago sativa* L.). *Australian Journal of Experimental Agriculture* **43**, 135-140.

Barclay Rm, Hebb DM, Brockwell J (1994) Differential colonisation of the roots of sown and volunteer annual species of *Medicago* in an acid soil. *Australian Journal of Experimental Agriculture* **34**, 745-752.

Bhatia CR, Maluszynski M, Nichterlein K and Van Zanten, L. 2001a. Grain legume cultivars derived from induced mutations and mutations affecting nodulation. *In*: Mutation Breeding. Pp. 1-44.

Black JN (1964) An analysis of the potential production of swards of subterranean clover (*Trifolium subterraneum* L.) at Adelaide, South Australia. *Journal of Applied Ecology* 1, 3-18.

Bolger TP, Pate JS, Unkovich MJ, Turner NC (1995) Estimates of seasonal nitrogen fixation of annual subterranean clover based pastures using the ^{15}N abundance technique. Plant and Soil 175, 57-66.

Bonish PM (1979) Clover rhizobia in soils: assessment of effectiveness using the plant infection count method. *New Zealand Journal of Agricultural Research* **22**, 89-93.

Bonython AL, Ballard RA, Charman N, Nichols PGH, Craig AD (2011) New strains of rhizobia that nodulate regenerating messina (*Melilotus siculus*) plants in saline soils. *Crop and Pasture Science* **62**, 427-436.

Bowman AM, Hebb DM, Munnich, DJ, Brockwell J (1998) *Rhizobium* as a factor in the re-establishment of legume based pastures on clay soils of the wheat belt of north-western New South Wales. *Australian Journal of Experimental Agriculture* **38**, 555-566.

Bowman AM, Smith W, Brockwell J (2004) Forecasting lucerne productivity under dryland farming conditions in central-western and western New South Wales. *Soil Biology and Biochemistry* **36**, 1253-1260.

Brockwell J (1975) Studies of field populations of *Rhizobium*: relationships between strain effectiveness, soil nitrogen levels, and response to applied nitrogen in subterranean clover pastures in north-eastern Victoria. *Field Station Record* **14**, 1-8.

Brockwell J (2001) *Sinorhizobium meliloti* in Australian soils: population studies of the root-nodule bacteria for species of *Medicago* in soils of the Eyre Peninsula, South Australia. *Australian Journal of Experimental Agriculture* **41**, 753-762.

Brockwell J, Bottomley PJ and Thies JE (1995) Manipulation of rhizobia microflora for improving legume productivity and soil fertility: A critical assessment. *Plant and Soil* **174**, 143-180.

Brockwell J, Fettell NA, Bowman AM, Smith W, Sweeney G, Charman N, Ballard RA (2008) Symbiotic competence of rose clover (*Trifolium hirtum All.*). *Australian Journal of Agricultural Research* **59**, 802-813.

Brockwell J, Gault RR, Peoples MB, Turner GL, Lilley DM, Bergersen FJ (1995) N_2 fixation in irrigated lucerne. *Soil Biology and Biochemistry* **27**, 589-594.

Brockwell J, Gault RR, Zorin M, Roberts MJ (1982) Effects of environmental variables on the competition between inoculum strains and naturalized populations of *Rhizobium trifolii* for nodulation of *Trifolium subterraneum* L. and on rhizobia persistence in the soil. *Australian Journal of Agricultural Research* **33**, 803-815.

Brockwell J, Hely FW (1966) Symbiotic characteristics of *Rhizobium meliloti*: an appraisal of the systematic treatment of nodulation and nitrogen fixation interactions between hosts and rhizobia of diverse origins. *Australian Journal of Agricultural Research* **17**, 885–899.

Brockwell J, Holliday RA, Daoud DM, Materon LA (1988) Symbiotic characteristics of a *Rhizobium* –specific annual medic, *Medicago rigidula* (L.) All. *Soil Biology and Biochemistry* **20**, 593-600.

Brockwell J, Holliday RA, Pilka A (1988) Evaluation of the symbiotic nitrogen-fixing potential of soils by direct microbiological means. *Plant and Soil* **108**, 163-170.

Brockwell J, Pilka A, Holliday RA (1991) Soil pH is a major determinant of the numbers of naturally occurring *Rhizobium meliloti* in non-cultivated soils in central New South Wales. *Australian Journal of Experimental Agriculture* **31**, 211–219.

Bullard GK, Roughley RJ, Pulsford DJ (2005) The legume inoculant industry and inoculant quality control in Australia: 1953-2003. *Australian Journal of Experimental Agriculture* **45**, 127-140.

Butler JHA (1988) Growth and N_2 fixation by field grown *Medicago littoralis* in response to added nitrate and competition from *Lolium multiflorum*. *Soil Biology and Biochemistry* **20**, 863-868.

Carroll BJ, Mathews A. 1990. Nitrate inhibition of nodulation in legumes. *In*: Molecular Biology of Symbiotic Nitrogen Fixation (Ed. PM Gresshoff). Pp. 159-180.

Carroll BJ, McNeil DL, Gresshoff PM (1985) A supernodulation and nitrate-tolerant (nts) soybean mutant. *Plant Physiology*. **78**, 34-40.

Casella S, Gault RR, Reynolds KC, Dyson JR, Brockwell J (1984) Nodulation studies on legumes exotic to Australia: *Hedysarum coronarium*. *FEMS microbiology letters* **22**, 37-45

Chapman DF and Caradus JR (1997) Effects of improved, adapted white clover (*Trifolium repens* L.) germplasm on the productive properties of a hill pasture. *New Zealand Journal of Agricultural Research* **40**, 207-221.

Charman N, Ballard RA. 2004. Burr medic (*Medicago polymorpha* L.) selections for improved N_2 fixation with naturalised soil rhizobia. *Soil Biology and Biochemistry* **36**, 1331-1337.

Charman N, Ballard RA, Humphries AW, Auricht GC (2008) Improving lucerne nodulation at low pH: contribution of rhizobial and plant genotype to the nodulation of lucerne seedlings growing in solution culture at pH 5. *Australian Journal of Experimental Agriculture* **48**, 512-517.

Chatel DL, Parker CA. 1973. Survival of field–grown rhizobia over the dry summer period in Western Australia. Soil Biology and Biochemistry 5, 415-423.

Chen H, Richardson AE, Gartner E, Djordjevic MA, Roughley RJ, Rolfe BG (1991) Construction of an acid tolerant *Rhizobium leguminosarum* biovar *trifolli* strain with enhanced capacity for nitrogen fixation. *Applied and Environmental Microbiology* 57, 2005-2011.

Chen J, Richardson AE, Rolfe BJ (1993) Studies on the physiological and genetic basis of acid tolerance in *Rhizobium leguminosarum* biovar *trifolii*. *Applied and Environmental Microbiology* **59**, 1798-1804.

Cocks PS (1980) Limitations imposed by nitrogen deficiency on the productivity of subterranean clover-based annual pasture in southern Australia. *Australian Journal of Agricultural Research* **31**, 95-107.

Coventry DR, Hirth JR Reeves TG (1985) Development of populations of *Rhizobium trifolii* and nodulation of subterranean clover following the cropping phase in crop- pasture rotations in southeastern Australia. *Soil Biology and Biochemistry* **17**, 17-22.

Coventry DR, Hirth JR, Fung KKH (1987) Nutritional restraints on subterranean clover grown on acid soils used for crop-pasture rotation. *Australian Journal of Agricultural Research* **38**, 163-76.

Dear BS, Li GD, Hayes RC, Hughes SJ, Charman N, Ballard RA (2007) *Cullen Australasicum* (syn. *Psoralea australasica*): a review and some preliminary studies related to its potential as a low rainfall perennial pasture legume. *The Rangeland Journal* **29**, 121-132.

Dear BS, Sandral GA, Peoples MB, Wilson BCD, Taylor JN, Rodham CA (2003) Growth, seed set and nitrogen fixation of 28 annual legume species on 3 Vertosol soils in southern New South Wales. *Australian Journal of Experimental Agriculture* **43**, 1101-1115.

Dear BS, Virgona JM, Sandral GA Swan AD, Morris S (2009) Changes in soil mineral nitrogen, nitrogen leached, and surface pH under annual and perennial pasture species. *Crop and Pasture Science* **60**, 975-986.

Denton MD, Coventry DR, Bellotti WD, Howieson JG (2000) Distribution, abundance and symbiotic effectiveness of *Rhizobium leguminosarum* bv. *trifolii* from alkaline pasture soils in South Australia. *Australian Journal of Experimental Agriculture* **40**, 25-35.

Denton MD, Coventry DR, Bellotti WD, Howieson JG (2011) Nitrogen fixation in annual *Trifolium* species in alkaline soils as assessed by the ^{15}N natural abundance method. *Crop and Pasture Science* **62**, 712-720.

Denton M, Hill CR, Bellotti WD, Coventry DR (2007) Nodulation of *Medicago truncatula* and *Medicago polymorpha* in two pastures of contrasting pH and rhizobial populations. *Applied Soil Ecology* **35**, 441-448.

Denton MD, Pearce DJ, Ballard RA, Hannah MC, Mutch LA, Norng S, Slattery JF (2009) A multi-site field evaluation of granular inoculants for legume nodulation. Soil Biology and Biochemistry 41, 2508-2516.

Dilworth MJ, Howieson JG, Reeve WG, Tiwari RP, Glenn AR (2001) Acid tolerance in root nodule bacteria and selecting for it. *Australian Journal of Experimental Agriculture* **41**, 435-446.

Drew EA, Ballard RA (2010) Improving N2 fixation from the plant down: compatibility of *Trifolium subterraneum* L. cultivars with soil rhizobia can influence symbiotic performance. *Plant and Soil* **327**, 261-277.

Drew EA, Charman N, Dingemanse R, Hall E, Ballard RA (2011) Symbiotic performance of Mediterranean *Trifolium* spp. with naturalised soil rhizobia. *Crop and Pasture Science* **62**, 903-913.

Drew EA, Gupta VVSR, Roget DK (2007) Herbicide use, productivity, and nitrogen fixation in field pea (*Pisum sativum*). *Australian Journal of Agricultural Research* **58**, 1204-1214.

Eberbach PL, Douglas LA (1989) Herbicide effects on the growth and nodulation potential of *Rhizobium trifolii* with subterranean clover. *Plant and Soil* **119**, 15-23.

Eberbach PL, Douglas LA (1991) Effect of herbicide residues in a sandy loam on the growth, nodulation and nitrogenase activity (C_2H_2/C_2H_4) of *Trifolium subterranean*. *Plant and Soil* **131**, 67-76.

Eckard RJ (1998) A critical review of research on the nitrogen nutrition of dairy pastures in Victoria. The University of Melbourne and Agriculture Victoria. ISBN 0 7311 4270 5. Pp.31.

Evans CM, Fettell NA, Brockwell J (2005a) Populations of *Sinorhizobium meliloti* congregate in the 30–60 cm section of the soil profile in a stand of dryland lucerne (*Medicago sativa*): is this where lucerne fixes its nitrogen? *Australian Journal of Experimental Agriculture* **45**, 225–230.

Evans J, Dear B and O'Conner GE (1990) Influence of an acid soil on the herbage yield and nodulation of five annual pasture legumes. *Australian Journal of Experimental Agriculture* **30**, 55-60.

Evans J, Hochman Z, O’Conner GE, Osbourne GJ (1988) Soil acidity and *Rhizobium*: their effects on nodulation of subterranean clover on the slopes of southern New South Wales. *Australian Journal of Agricultural Research* **38**, 605-618.

Evans PM, Howieson JG, Nutt BJ (2005b) Increased yield and persistence of several annual medic species and *Medicago sativa* by inoculation with selected strains of *Sinorhizobium meliloti* and *S. medicae. Australian Journal of Experimental Agriculture* **45**, 217–224.

Ewing MA, Robson AD (1990) The effect of early season nitrogen supply on the early growth and nodulation of several annual *Medicago* species. *Australian Journal of Agricultural Research* **41**, 489-497.

Farquharson RL (2009) The impact of acetohydroxyacid synthase inhibiting herbicides on symbiotic nitrogen fixation of grain and pasture legumes. PhD Thesis. The University of Adelaide. Pp. 251.

Gault RR, Pilka A, Hebb DM, Brockwell J (2004) Nodulation studies of legumes exotic to Australia: symbiotic relationships between *Chamaecytisus palmensis* (tagasaste) and *Lotus* spp. *Australian Journal of Experimental Agriculture* **34**, 385-394.

Gault RR, Peoples MB, Turner GL, Lilley DM, Brockwell J, Bergersen FJ (1995) Nitrogen fixation by irrigated lucerne during the first three years after establishment. *Australian Journal of Agricultural Research* **46**, 1401-1425.

Gemell LG, Hartley EJ, Herridge DF (2005) Point-of-sale evaluation of preinoculated seed and custom-inoculated pasture legume seed. *Australian Journal of Experimental Agriculture* **45**, 161-169.

Gemell LG, Roughley RJ (1993) Field evaluation in acid soils of strains of *Rhizobium leguminosarum* bv. *trifolii* selected for their tolerance or sensitivity to acid soil factors in agar medium. *Soil Biology and Biochemistry* **10**, 1447-1452.

Gibson AH (1968) Nodulation failure in *Trifolium subterraneum* L. Woogenellup (Sum. Marrar). *Australian Journal of Agricultural Research* **19**, 907-918.

Gibson AH, Curnow BC, Bergersen FJ, Brockwell J, Robinson AC (1975) Studies of field populations of Rhizobium: Effectiveness of strains of *Rhizobium trifolii* associated with *Trifolium subterraneum* L. pastures in south-eastern Australia. *Soil Biology & Biochemistry* **7**, 95-102.

Gibson AH, Date RA, Ireland JA, Brockwell J (1976) A comparison of competitiveness and persistence amongst five strains of *Rhizobium trifolii*. *Soil Biology and Biochemistry* **8**, 395-401.

Good AG, Beatty PH (2011) Fertilising Nature: A tragedy of excess in the commons. *PLoS Biol* **9**, 1-15.

Hartley E, Gemell G, Herridge DF (2004) Lime pelleting inoculated serradella (*Ornithopus* spp.) increases nodulation and yield. *Soil Biology and Biochemistry* **36**, 1289-1294.

Herridge DF, Roughley RJ, Brockwell J. 1984. Effect of soil nitrate on the establishment and functioning of soybean symbiosis in the field. *Australian Journal of Agricultural Research*. 35, 149-161.

Hill R 1936. Subterranean Clover, its History in South Australia, J. Dept. Agric., Sth. Aust., 40, pp. 322-330.

Hill MJ, Donald GE (1998) Australian Temperate Pastures Database. National Pastures Improvement Coordinating Committee.

Howie JH, Ballard RA, deKoning C, Sandral G, Charman N (2001) *Trigonella balansae*- a new pasture legume for the alkaline soils of southern Australia. In: Proceedings of 10th Australian Agronomy Conference, Hobart.

Howie JH, Bell CA (2005) Field performance of an annual medic tolerant of sulfonylurea herbicide residues. In: Proceedings of the 20th International Grasslands Congress, Dublin, Ireland.

Howieson J, Ballard RA (2004) Optimising the legume symbiosis in stressful and competitive environments within southern Australia — some contemporary thoughts. Soil Biology and Biochemistry 36, 1261–1273.

Howieson JG, Ewing MA (1986) Acid tolerance in the *Rhizobium meliloti-Medicago* symbiosis. *Australian Journal of Agricultural Research* **37**, 55–64.

Howieson JG, Nutt B, Evans P (2000) Estimation of host-strain compatibility for symbiotic N-fixation between *Rhizobium meliloti*, several annual species of *Medicago* and *Medicago sativa*. Plant and Soil 219, 49-55.

Howieson JG, Yates R, O'Hara GW, Ryder M, Real D (2005) The interaction of *Rhizobium leguminosarum* biovar *trifolii* in nodulation of annual and perennial *trifolium* spp. from diverse centres of origin. *Australian Journal of Experimental Agriculture* 45: 199-207.

Howieson JG, Ballard RA, Yates RJ, Charman N (2011) Selecting improved *Lotus* nodulating rhizobia to expedite the development of new forage species. *Plant and Soil* **348**, 231-243.

Howieson JG, Ewing MA (1989) Annual species of *Medicago* differ greatly in their ability to nodulate on acid soils. *Australian Journal of Agricultural Research* **40**, 843–850.

Howieson JG, Robson AD, Abbott LK (1992) Acid-tolerant species of *Medicago* produce root exudates at low pH which induce the expression of nodulation genes in *Rhizobium meliloti*. *Plant Function and Evolutionary Biology* **19**, 287-296.

Ireland JA, Vincent JM (1968) A quantitative study of competition for nodule formation. *In*: Transactions 9th International Congress on Soil Science. Adelaide, Australia. Vol2, pp.85-93.

Jensen ES, Hauggaard-Nielsen H (2003) How can increased use of biological fixation in agriculture benefit the environment? *Plant and Soil* **252**, 177-186.

Kidd CR, Murray GM, Pratley JE, Leys AR (2002) Effect of timing of pasture grass removal on subsequent take-all incidence and yield in wheat in southern New South Wales. *Australian Journal of Experimental Agriculture* **42**, 1087-1094.

Kishinevsky BD, Sen D, Yang G (1996) Diversity of rhizobia isolated form various *Hedysarum* species. *Plant and Soil* 186, 21-28

Koopman DJ, Tow PG, Reeves TG, Gibson AH (1995) Soil acidification, chlorsulfuron application and *Rhizobium meliloti* as factors in lucerne yield decline. *Soil Biology and Biochemistry* **27**, 673-677.

McInnes A. (2002) Field Populations of Bradyrhizobia Associated with Serradella. PhD Thesis. University of Western Australia. 229 pp.

Mundy GN, Jones HR and Mason WK (1988) Nitrogen fixation activity by white clover pastures during flood irrigation cycles. *Australian Journal of of Agricultural Research* **39**, 409-401.

Munday GN (1999) A review of nitrogen research with irrigated pastures in northern Victoria. Department of Natural Resources and Environment, Victoria.

Munns DN (1968) Nodulation of *Medicago sativa* in solution culture. *Plant and Soil* **28**, 129–146.

Mytton LR , Rys GJ (1985) The potential for breeding white clover (*Trifolium repens* L.) with improved nodulation and nitrogen fixation when grown with combined nitrogen. 2. Assessment of genetic variation in *Trifolium repens*. *Plant and Soil* **88**, 197-211.

Nandasena KG, O'Hara GW, Tiwari RP, Sezmis E, Howieson JG (2007a) In situ lateral transfer of symbiosis islands results in rapid evolution of diverse competitive strains of mesorhizobia suboptimal in symbiotic nitrogen fixation on the pasture legume *Biserrula pelcinus* L. *Environmental Microbiology* **9**, 2496-2511.

Nandasena KG, O'Hara GW, Tiwari RP, Willems A, Howieson JG (2007b) *Mesorhizobium ciceri* biovar *biserrulae*, a novel biovar nodulating the pasture legume *Biserrula pelecinus* L. *International Journal of Systematic and Evolutionary Biology* **57**, 1041-1045.

Nair RM, Howie J, Ballard RA, Hutton RE, Charman N, Preston C (2004) Genetic improvement of strand medic (*Medicago littoralis* Rohde ex Lois.) for Australian farming systems. *In:* New Directions for a Diverse Planet. Proceedings of the 4th International Crop Science Congress. Brisbane, Australia.

Nichols P, Craig A, Bonython A, Rodgers MJ, Ballard R, Charman N, Hughes S, Colmer T, McClements D, Barrett-Lennard E (2010) Development of *Melilotus siculus* – a new salt tolerant and water-logging tolerant annual fodder legume species for Mediterranean-type climates. *In:* Sustainable Use of Genetic Diversity in Forage and Turf Breeding. (Ed. C Huyghe). Springer Science. Pp. 131-135.

NLAWRA (2001) Nutrient balance in regional farming systems and soil nutrient status. National Land and Water Resources Audit. (www.anra.gov.au).

O'Hara, GW, Boonkerd N, Dilworth MJ (1988) Nutritional constraints to nitrogen fixation. *Plant and Soil* **108**, 93-110.

Peoples MB, Baldock JA (2001) Nitrogen dynamics of pastures: nitrogen fixation inputs, the impact of legumes on soil fertility, and the contributions of fixed nitrogen to Australian farming systems. *Australian Journal of Experimental Agriculture* **41**, 327-346.

Peoples MB, Gault RR, Scammell GJ, Dear BS, Virgona J, Sandral GA, Paul J, Wolfe EC, Angus JF (1998) Effect of pasture management on the contributions of fixed N to the economy of ley farming systems. *Australian Journal of Agricultural Research* **49**, 459-74.

Puckridge DW and French RJ (1983) The annual legume pasture in cereal-ley farming systems of southern Australia: A review. *Agriculture, Ecosystems and Environment* **9**, 229-267.

Quigley PE, Cunningham PJ, Hannah M, Ward GN, Morgan T (1997) Symbiotic effectiveness of *Rhizobium leguminosarum* bv. *trifolii* collected from pastures in south-western Victoria. *Australian Journal of Experimental Agriculture* **37**, 623-630.

Reeve WG, Brau L, Castelli J, Garau G, Sohlenkamp C, Geiger O, Dilworth MJ, Glenn AR, Howieson JG, Tiwari RP (2006) The *Sinorhizobium medicae* WSM419 lpiA gene is transcriptionally activated by FsrR and required to enhance survival in lethal acid conditions. *Microbiology* **152**, 3049-3059.

Reeve W, Chain P, O'Hara G, Ardley J, Nandesena K, Brau L, Tiwari R, Malfatti S, Kiss H, Lapidus A, Copeland A, Nolan M, Land M, Hauser L, Chang YJ, Ivanova N, Mavromatis K, Kyrpides N, Gollagher M, Yates R, Dilworth M, Howieson, J (2010a) Complete genome sequence of the *Medicago* microsymbiont *Ensifer* (*Sinorhizobium*) *medicae* strain WSM419. *Standards in Genomic Sciences* **2**, 77-86.

Reeve W, O'Hara G, Chain P, Ardley J, Brau L, Nandesena K, Tiwari R, Copeland A, Nolan M, Han C, Brettin T, Land M, Ovchinikova G, Ivanova N, Mavromatis, Markowitz V, Kyrpides N, Melino V, Denton M, Yates R, Howieson, J (2010b) Complete genome sequence of *Rhizobium leguminosarum* bv. *trifolii* strain WSM1325, an effective microsymbiont of annual Mediterranean clovers. *Standards in Genomic Sciences* **2**, 347-356.

Richardson AE, Henderson AP, Kames GS, Simpson RJ (1988a) Consequences of soil acidity and the effect of lime on the nodulation of *Trifolium subterraneum* L. growing in an acid soil. *Soil Biology and Biochemistry* 20, 439-445.

Richardson AE, Simpson RJ (1988) Enumeration and distribution of *Rhizobium trifolii* under a subterranean clover-based pasture growing in an acid soil. *Soil Biology and Biochemistry* **20**, 431-438.

Richardson AE, Simpson RJ (1989) Acid tolerance and symbiotic effectiveness of *Rhizobium trifolii* associated with *Trifolium subterraneum* L. based pasture growing in an acid soil. *Soil Biology and Biochemistry* **21** 87-95.

Richardson AE, Simpson RJ, Djordjevic MA, Rolfe BG (1988b) Expression of nodulation genes in *Rhizobium leguminosarum* biovar *trifolii* is affected by low Ph and by Ca and Al ions. *Applied and Environmental Microbiology* **54**, 2541-2548.

Riffkin PA, Quigley PE, Cameron FJ, Gault RR, Peoples MB, Thies JE (1999a) Annual nitrogen fixation in grazed dairy pastures in south-western Victoria. *Australian Journal of Agricultural Research* **50**, 273-81.

Riffkin PA, Quigley PE, Kearney GA, Cameron FJ, Gault RR, Peoples MB, Thies JE (1999b) Factors associated with biological Nitrogen Fixation in dairy pastures in south-western Victoria. *Australian Journal of Agricultural Research* **50**, 261-72.

Roberston M (2006) Lucerne prospects: Drivers for the widespread adoption of lucerne for profit and salinity management. Cooperative Research Centre for Plant- based management of Dryland Salinity, Perth.

Roughley RJ, Gemell LG, Thompson JA, Brockwell J (1993) The number of *bradyrhizobium* sp. (*Lupinus*) applied to seed and its effect on rhizosphere colonisation, nodulation and yield of lupin. *Soil Biology and Biochemistry* **25**, 1453-1458.

Sandral GA, Dear BS, Pratley JE, Cullis BR (1997) Herbicide dose rate response curves in subterranean clover determined by a bioassay. *Australian Journal of Experimental Agriculture* **37**, 67-74.

Sandford P, Pate JS, Unkovich MJ (1994) A survey of the proportional dependence of subterranean clover and other pasture legumes on N_2 fixation in south-west Australia utilizing ^{15}N natural abundance. *Australian Journal of Agricultural Research* **45**, 165-181.

Sandford P, Pate JS, Unkovich MJ, Thompson AN (1995) Nitrogen fixation in grazed and ungrazed subterranean clover pasture in south west Australia assessed by the ^{15}N natural abundance technique. *Australian Journal of Agricultural Research* **46**, 1427-1443.

Schnabel E, Mukherjee A, Smith L, Kassaw T, Long S, Frugoli J. 2010. The *lss* supernodulation mutant of *Medicago truncatula* reduces expression of the *SUNN* gene. *Plant Physiology*. 154, 1390-1402.

Scott BJ, Ridley AM, Conyers MK (2000) Management of subsoil acidity in long term pastures of south eastern Australia: a review. *Australian Journal of Experimental Agriculture*. 40, 1173-1198.

Sitepu S (2001) Selection of Balansa clover lines using naturalised soil rhizobia. Masters Thesis, University of Adelaide.

Slattery JF, Coventry DR (1993) Variation of soil populations of *Rhizobium leguminosarum* bv. *trifolii* and the occurrence of inoculant rhizobia in nodules of subterranean clover after pasture renovation in north-eastern Victoria. *Soil Biology & Biochemistry* **25**, 1725-1730.

Slattery JF, Coventry DR (1995) Acid tolerance and symbiotic effectiveness of *Rhizobium Leguminosarum* bv. *trifolii* isolated from subterranean clover growing in permanent pastures. *Soil Biology and Biochemistry* **27**, 111-115

Slattery JF, Coventry DR (1999) Persistence of introduced strains of *Rhizobium leguminosarum* bv *trifolii* in acidic soils of north-eastern Victoria. *Australian Journal of Experimental Agriculture* **39**, 829-837.

Slattery JF, Coventry DR, Slattery WJ (2001) Rhizobial ecology as affected by soil environment. *Australian Journal of Experimental Agriculture* **41**, 289-298.

Stepkowski T, Moulin L, Krzyzanska A, McInnes A, Law IJ, Howieson JG (2005) European origin of *Bradyrhizobium* populations infecting lupins and serradella in soils of western Australia and South Africa. *Applied and Environmental Microbiology* **71**, 7041-7052.

Sullivan JT and Ronson CW (1998) Evolution of rhizobia by acquisition of a 500-kb symbiosis island that integrates into a phe-tRNA gene. *Proc. Nat. Acad. Sci. USA*. **95**, 5145-5149.

Terpolilli JJ, O'Hara GW, Tiwari RP, Dilworth MJ, Howieson JG (2008) The model legume *Medicago truncatula* A17 is poorly matched for N_2 fixation with the sequenced microsymbiont *Sinorhizobium meliloti* 1021. *New Phytologist* **179**, 62-66.

Thies JE, Singleton PW, Bohlool BB (1991) Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. *Applied and Environmental Microbiology* **57**, 19-28.

Unkovich M, Baldock J (2008) Measurement of asymbiotic N_2 fixation in Australian agriculture. *Soil Biology and Biochemistry* **40**, 2915-2921.

Unkovich MJ, Baldock J and Peoples MB (2010) Prospects and problems of simple linear models for estimating symbiotic N2 fixation by crop and pasture legumes. *Plant and Soil* **329**, 75-89.

Unkovich MJ and Pate JS (1998) Symbiotic effectiveness and tolerance to early season nitrate in indigenous populations of subterranean clover rhizobia from S.W. Australian pastures. *Soil Biology and Biochemistry* **30**, 1435-1443.

Unkovich MJ, Pate JS and Sanford P (1997) Nitrogen fixation by annual legumes in Australian Mediterranean agriculture. *Australian Journal of Agricultural Research* **48**, 267-93.

Watkin ELJ, O'Hara G, Glen AR (2000) Identification of tolerance to soil acidity in inoculant strains of *Rhizobium leguminosarum* bv. *trifolii*. *Soil Biology and Biochemistry* **32**, 1393-1403.

Watkin ELJ, O'Hara G, Glen AR (2003) Physiological responses to acid stress of an acid-soil tolerant and an acid-soil sensitive strain of *Rhizobium leguminosarum* biovar *trifolii*. *Soil Biology and Biochemistry* **35**, 621-624.

Yates R, Foster K, Nichols P, Ewing M (2006) Flamenco - a new variety of sulla for southern Australia. In: Proceedings of the 13th Australian Agronomy Conference. Perth.

Yates RJ, Howieson JG, Reeve WG, Brau L, Speijers J, Nandasena K, Real D, Sezmis E, O'Hara GW (2008) Host-strain mediated selection for an effective nitrogen-fixing symbiosis between *Trifolium* spp. and *Rhizobium leguminoasrum* biovar *trifolii*. *Soil Biology and Biochemistry* **40**: 822-833.

Young RR (1988) Build up of soil nitrogen under pastures. *In:* Proceedings of the Riverina Outlook Conference. Wagga Wagga, NSW.

8. Appendix 4

Invertebrate Pests of Major Pasture Species

Maarten Ryder
February 2012

8.1 Introduction

Much of the research effort directed at invertebrate pests of pastures in southern Australia finished in the mid 1990s. This has resulted in loss of expertise in the biology and control of pasture pests. The main exception is that research on pests of pasture legumes grown in the rotational systems of the pasture – cropping zone has continued, and important advances have been made in this area.

Since the mid 1990s, agricultural systems have continued to change towards minimum tillage and stubble retention (or conservation farming) in the pasture – cropping zone. The evolution of management systems has resulted in changes in the pest status of some invertebrates: some may have become less important, but other potential new pests are emerging (Lawrence, 2009). For example, pasture pests that are favoured by minimum tillage and stubble retention include Bryobia pasture mites, slugs, snails and pasture webworm (Bailey, 2007). Some of the new threats need to be dealt with quickly as they are clearly capable of causing large losses in production; others need to be assessed to determine what action(s) should be taken.

8.2 Production losses caused by pests of pastures.

It seems that it is more than 20 years since an overall assessment was made of the losses caused by weeds, pests and diseases of pastures (Sloane et al., 1988; **note** that this study was based on a relatively small sample of grazing properties). It would be prudent for the various industries that rely on pastures for animal production (Meat, Dairy, Wool) to consider a fresh evaluation, since production systems and the industries themselves have changed considerably in the intervening time.

In the preparation of this report, 51 papers and reports about “important” invertebrate pests of pastures were consulted with a specific focus on finding information about economic losses or losses in productivity (either herbage or stock productivity). Only 11 papers reported on the collection of data on losses due to pests. The remaining papers made brief statements about pests being “important”, “serious” or “key”, usually with no reference to estimated losses to industry, either decreased herbage or stock production or economic loss. Occasionally, reference was made to money spent by growers nationally on chemical pesticides as an indication of the importance of pests.

Papers and reports which have dealt with assessment of economic loss or lost production are:
* Roberts and Ridsdill-Smith (1979). Damage caused by Pasture scarab, *Sericesthis geminata,* in northern NSW led to a 50% decrease in sheep production at lower stocking rates.

* Sloane et al. (1988): weeds, pests and diseases nationally in relation to sheep grazing for wool; estimated that Redlegged Earth Mite (RLEM), Lucerne Flea (LF) and Bluegreen Aphid (BGA) caused $228 million p.a. loss to the wool industry.

* Allen et al. (1989) reported that pest damage caused by RLEM, LF and BGA led to up to 90% reduction in seed yield in medic pastures in lower rainfall areas of SA.

* Ridsdill-Smith (1991). The effect of RLEM alone on pastures was estimated to cause $56 – 112 million p.a. lost production to the wool industry and $81 million p.a. to the beef industry, using data for 1989/1990.

* Pauley and Miller (1993) made a detailed analysis of losses caused by Corbies and Cockchafers in Tasmanian pastures. They estimated that combined losses from Corbies, Winter Corbies, Blackheaded Cockchafer (BHC) and Redheaded Cockchafer (RHC) ranged from $2.2 million in a season of above average rainfall, to $6 million in an average season. Total area infested by the four pests was estimated to be nearly 120,000 ha. The situation is very likely to have changed in the intervening 20 years. For example, RHC is now estimated to affect 160,000 ha which is 10x the area infested in the early 1990s (Pauley and Miller, 1993).

* Berg et al. (1993) reported that Redheaded Cockchafer caused losses of up to $100/ha in infested fat lamb production areas and up to $500/ha in infested dairying areas.

* Hopkins et al. (1994) calculated that lost medic production, due mainly to Spotted Alfalfa Aphid (SAA) in a good growing season, was enough to support an extra 5 to 7 DSE per hectare. In addition, damage to medic pastures due to RLEM, LF and BGA caused reduction in seed set of between 15 – 70% over three years of field trials.

* McDonald et al (1994) and Nutt et al (1994). Aphid-tolerant barrel medic varieties supported 60% greater wool production than an aphid-susceptible variety (‘Cyprus’) in the northern wheatbelt of WA. In addition, the aphid tolerant cultivar Parabinga had a 3-fold higher seed reserve compared to the highly susceptible ‘Cyprus’ at the same stocking rate over 4 years. Improved persistence was also achieved with ‘Parabinga’ at a 60% higher stocking rate.

* Young et al. (1995) estimated that controlling RLEM led to a $49 / ha increase in income from grazing a clover pasture in the Great Southern region of WA, excluding the cost of control, and suggested that losses estimated by Sloane et al (1988) were probably underestimates.

* Ridsdill-Smith (1997). This review contains several different estimates of lost production and impact of Redlegged Earth Mite (RLEM). These include: a 12% loss of wool production during summer after feeding by RLEM in spring; up to 50% lost seed production in annual pasture legumes; an estimate of the “stocking rate” of RLEM “biomass” per hectare of 0.5 – 1 dry sheep equivalent (DSE), which represented approximately 7–8% of the potential sheep stocking rates in the pasture.

Apart from causing direct losses in production, the activity of serious pests can lead to dramatic changes in pasture composition, with increases in weedy species. The benefits of controlling pests include retaining a better pasture composition.

2. In dealing with damage to pastures caused by invertebrate pests, we need to keep in mind the differential effects that pests can have on the various stages of pasture growth, i.e. the effects of pests on seed germination and seedling establishment, effects on vegetative growth (particularly spring growth) and on flowering and seed set, which is critical for managing pastures that rely on regeneration from seed (Allen et al., 1989; Ridsdill-Smith, 1997). Assessment of pest damage should include effects on seed set.

3. There is a strong interaction between grazing management and the control of invertebrate pests and this needs to be taken into account in the design of research programs as well as the application of research results to production systems (Roberts and Ridsdill-Smith, 1979; Roberts and Morton, 1985; Grimm et al., 1995). For example, Grimm et al. (1995) and Michael et al. (1997) showed that intensive grazing to predetermined amounts of feed on offer in spring gave good control of insect pests including RLEM, and this effect carried over into the following season. On the other hand, set stocking regimes gave very different results depending on whether or not chemical pest control treatments were applied. Set stocking with pest control gave the highest amounts of dry matter, and the best conversion of rainfall to pasture growth, whereas set stocking without pest control gave the lowest values (approx. half the amount of dry matter in some cases). The extra feed available as a result of pest control was largely unused, however. This raises the important point that when pests are controlled and pasture production increased as a result, this should preferably be incorporated into a grazing management system that can use the extra feed that has been generated. Hyder et al (2003) continued this theme of grazing management to improve productivity, with pest control as an important factor in the system.

4. All pests are patchy in occurrence, and soil type as well as climate will greatly influence pest populations and therefore impact on pastures. For example, Redlegged Earth Mite favours sandy soils whereas Lucerne Flea is more suited to heavier textured soils. Pasture scarabs and crickets are also more prevalent on particular soil types. For example, crickets are a problem only on cracking clay soils. In Tasmania, Redlegged Earth Mite is found more commonly on lighter soils which occur in coastal areas, whereas BOM is more widespread across the agricultural regions of the state (McQuillan et al., 2007). Because of the strong interaction between soil type and the incidence and severity of pests, it is important for researchers and consultants to collect information about soil type when recording pests and pest damage to pastures.

5. Locusts can have substantial impacts on pasture production in particular years and seasons, but these pests have not been included in this report. The Australian Plague Locust Commission deals with the control of locusts, including a responsibility for monitoring, forecasting, research and control measures.

In **Section 2**, major pests of pastures in southern Australia are described, together with selected recent research results on pest control.

Section 3 gives details about the most important pasture pests, subdivided by type of pasture plant. These are in the order Annual Clovers, White Clover and other perennial Clovers, Annual

Medics, Lucerne, Other pasture legumes and Sown Grasses. Information about the most important pests and their rankings comes from both the scientific literature and from the recent survey of agricultural consultants (see Appendix 2).

8.3 Descriptions of major pests, damage caused to pastures and selected recent research results on pest control

The descriptions have been adapted from Pavri (2007), Pavri & Young (2007) and McQuillan et al (2007) and additional information from selected recent research on pest biology and control has been added.

8.3.1 Mites

Redlegged Earth Mite (Halotydeus destructor, RLEM) is a very serious introduced pest of all pastures and field crops, and is especially damaging to seedlings (Ridsdill-Smith, 1997). Various estimates of lost productivity range from $130 to $200 million p.a. (Ridsdill-Smith, 1991). Annual cost to the industry in Tasmania alone was suggested to be several million dollars (McQuillan et al., 2007). RLEM is active in cool winter months, and more commonly attacks the legume component of pastures. After hatching in autumn, the mite passes through approx 3 generations (8 wk per generation on average). Monitoring for mite activity at the seedling stage is critical.

Controls: there are legume varieties which are less susceptible to RLEM (Nichols 2010, 2011a, 2011b). Seed treatment with systemic pesticide can be used at sowing time. Timing of spray treatment is important: spraying in spring before adults produce oversummering eggs is most effective and increases seed yield (Timerite®, Ridsdill-Smith et al, 2008). Other controls include control of broad-leaved weeds and heavy grazing in spring, though this may not be appropriate for all pastures in all situations. For example, subclovers are more grazing tolerant than medics and in medium/high rainfall have a greater opportunity to recover than do medics in a more variable low rainfall environment. James (1995) suggested that natural enemies could have an important role in the control of earth mites. The French predatory mite, *Anystis wallacei*, has been introduced as a biological control agent for RLEM, but this predator is slow to establish and disperse (Umina, 2007a).

Blue Oat Mite (Penthaleus species, BOM) is similar in appearance to RLEM, but BOM have a red dot on their backs. BOM are a widespread, serious pest of pastures. They more commonly attack grasses and cereals (see McQuillan et al., 2007) but will also feed on legumes and weeds. BOM are active in cooler winter months and are especially damaging at germination. After hatching in autumn, BOM pass through about 2 generations of 11 – 12 weeks. Monitoring for mite activity at the seed germination stage is critical. Control options include foliar applications of pesticides, which is more effective in autumn early after the break of season. Spring spraying is relatively ineffective. Control via grazing management includes heavy grazing in winter and spring. Natural enemies have been introduced with limited success.

BOM is a serious pest in Northern NSW (north of Armidale) and SE Queensland. RLEM becomes patchy north of Dubbo and is not generally found north of Gunnedah (A. Nicholas, pers. comm.).

Hill et al. (2011) recently mapped the distribution of the three species of *Penthaleus* (*P. major, P. falcatus* and *P. tectus*) that occur in Australia. All three species are very similar in appearance, however they show clear differences in biology and ecology. Although these differences need to be taken into account when planning control strategies with crops, there is currently less urgency in relation to pasture management (P. Umina, pers. comm. Feb 2012). *P. major* is widespread across the southern regions of Australia (WA, SA, Vic, NSW, Tas). Hill et al. (2011) reported *P. falcatus* and *P. tectus* from southwestern WA for the first time.

Bryobia mites and Balaustium mite Arthur et al. (2010) and Arthur et al. (2011) documented the occurrence and described some of the biology of these pest mites which are an emerging problem in agriculture in southern Australia. These mites will be a challenge to control as they have relatively high levels of natural resistance to chemical pesticides (Arthur et al., 2010). Four species of *Bryobia* have been found in Australia and these are more widely distributed across southern Australia than *Balaustium medicagoense*. Both genera of mites occur mainly in areas with a Mediterranean climate. From an extensive field survey, *Bryobia* spp. were commonly recovered from pasture plants, preferring lucerne and clover, and weeds (wild radish, barley grass and capeweed). *Ba. medicagoense* tended to favour grasses over clovers but was also often found on lucerne, clover and weeds including barley grass and capeweed.

Arthur et al. (2011) reported that *Ba. medicagoense* appeared to go through two generations per annum with an active period from March to December and a diapause or dormant period in summer. The situation with *Bryobia* appeared to be more complex with four species and differences between species in their annual life cycles. For example, whereas one *Bryobia* species went through 2 generations per year (active period from March – December with diapause in summer), another species had four generations per year with no diapause stage.

8.3.2 Springtails

Lucerne Flea (Sminthurus viridis, LF) is a serious, widespread pest of pastures across southern Australia. LF attacks a wide range of pasture legume species and can also be a pest of ryegrass. Depending on temperature, LF can go through up to 6 generations through late autumn, winter and spring. LF tends to be a more important pest in areas with heavier clay soils. Regular monitoring is necessary and there is an action threshold for leaf damage level (Pavri, 2007).

Controls: seed treatment and also spray treatment 3 weeks after the autumn hatch, with possible follow-up spray, can be used. Heavy grazing needs to be combined with chemical spray. Natural enemies: pasture snout mite and spiny snout mite are predators which have been introduced in eastern Australia and WA. Spiny snout mite (*Neomolgus capillatus*) gave effective control of LF in autumn in Tasmania, but spring populations of LF were still at damaging levels (Ireson and Webb, 1995). Recent research (Roberts et al., 2011b) reported that pasture snout mite (*Bdellodes lapidaria*) did not reduce spring populations of LF in the field in Victoria. In another study, Roberts et al. (2011a) found that soil moisture and temperature related strongly to autumn emergence of LF and they suggested that pest pressure in autumn could be predicted. They also found that oversummering eggs were produced through the winter by several generations of females, and that current late-season spraying strategies may not be as effective against LF as previously thought. Nevertheless, a spring spraying would still reduce the production of oversummering eggs by the last generation of females.

8.3.3 Aphids

Bluegreen Aphid (BGA), Cowpea Aphid (CPA), Pea Aphid (PA), Spotted Alfalfa Aphid (SAA)

Several species of aphid are serious pests of pasture plants in southern Australia. Some of these aphid species are similar in appearance, making them hard to distinguish. BGA, CPA and PA can all cause serious damage to a range of introduced pasture legumes. SAA occurs two forms in Australia: form *maculata* prefers lucerne whereas the other prefers clovers. Aphids are particularly damaging to seedlings, during autumn, spring and in cooler moister summers.

The apparently accidental introduction of BGA and SAA into Australia in the 1970s caused widespread, serious economic losses in legume pastures (as well as in susceptible crops). The selection and breeding of aphid-resistant cultivars of pasture legumes resulted in better survival and productivity (Howie, 1998). However, aphids can develop new biotypes which overcome plant resistance, especially if this is single gene resistance. The recent appearance of a new biotype of BGA that has overcome plant resistance (Humphries et al., 2010) could have very damaging consequences.

Different species of pasture legumes have varying levels of resistance to the most important aphids. There is also considerable variation in resistance among cultivars (PIRSA, 2004). Screening and breeding for aphid resistance in subclover, medic, lucerne and other legumes is a continuing goal of research to improve pastures (Nair et al., 2007; Rose, 2008).

Chemical control of aphids is not likely to be economic except for seed crops (Pavri, 2007). Sowing resistant or tolerant species and varieties is the main control option. Avoiding the use of broad-spectrum insecticides can encourage natural enemies.

Aphids also play a very important role as vectors that spread plant viruses in pastures and crops (see Appendix 5 by Barbetti and Jones).

A considerable research effort nationally is directed at aboveground aphids and their control, in pastures (Edwards et al., 2008), broad acre crops and vegetables.

Root Aphid

Root Aphid (*Aploneura lentisci*) is not mentioned in Bailey (2007), however this aphid is considered by some to be an <u>emerging pest</u>, particularly of ryegrass in higher rainfall areas. In both Australia and New Zealand, Root Aphid is found on the roots of ryegrass and tall fescue. Further research is needed on the biology and distribution of Root Aphid in Australia, and on the impact of this pest on pasture production levels. Research from New Zealand suggests that certain fungal endophytes of ryegrass can reduce populations of Root Aphids and increase plant growth compared to plants without endophytes (Popay and Gerard, 2007). Endophyte-containing ryegrasses are evidently being trialled in Victoria (J. Ridsdill-Smith, pers. comm., Feb. 2012).

8.3.4 Weevils

Weevils can be difficult pests to control. In the southern Australian environment, their life cycles are long, and they can be present, undetected, as small larvae for periods of more than a year (J. Ridsdill-Smith, pers. comm. Feb 2012).

Sitona Weevil (Sitona discoideus)

Sitona Weevils attack legumes including lucerne, subclover and medics. Sitona Weevil larvae live in the soil and feed on roots of the host plant. Damage caused by larvae can reduce symbiotic N fixation. Adults can cause significant damage to above ground parts of the plant early in the season.

Chemical control may be needed if adults are killing seedlings in autumn. A weevil parasitoid, *Microtonus aethiopoides*, has been introduced into Australia as a biocontrol agent but appears to have had only a limited effect in reducing Sitona damage. The biocontrol approach using *M. aethiopoides* has been more successful in New Zealand, against *S. discoideus* and *S. lepidus* (clover root weevil, NZ only) possibly because of climate difference, and the parasitoid completes more generations in summer in New Zealand than in Australia (Kean and Barlow, 2001; Goldson et al., 2005). There is some debate about the seriousness of Sitona Weevil as a pest of pastures in Southern Australia.

Whitefringed Weevils (Naupactus leucoloma, previously called Graphognathus leucoloma, and N. peregrinus, WFW)

The larvae of Whitefringed Weevils live in the soil during winter and spring, and the adults, which do not fly, emerge in summer. WFW has been a major pest of maize in Queensland and is now considered to be an emerging pest of pastures in southeastern Australia. WFW often have one generation each year, but the life cycle can take two years in southern Australia. WFW are similar in appearance to Sitona Weevil. They have a wide host range, including clovers, medics and lucerne, and most damage occurs in summer and autumn. Lucerne is a particularly good host of WFW (Barnes and de Barro, 2009), and can be seriously damaged at the seedling stage by larval feeding on the roots. Feeding on lucerne in summer by adults is not considered important.

Chemical control of adults is only economic in higher value crops. Cultural controls include rotation with a non-host in the pasture-cropping zone and avoidance of planting lucerne in or near areas that have been recently affected. Biological control: entomopathogenic nematodes (*Heterorhabditis*) were reported to attack WFW with substantial impact on growth of 2-year old irrigated lucerne in the field in Victoria (Sexton and Williams 1981). This study has been frequently referred to in the literature, but has not been developed to commercial application.

Small Lucerne Weevil

Small Lucerne Weevil occurs in WA, where it can be a serious problem on lucerne and subclover, and in NSW. Pastures are most at risk in autumn and in summer. Larvae feed on the roots and adults on the emerging seedlings (cotyledons). Chemical control can be used (2 applications in late summer / autumn) but timing is critical: adults need to be treated before egg laying. Cultural control: rotation with a non-host (e.g. cereals) with pastures can be effective. Natural enemies have not been reported.

Mandalotus Weevil

Mandalotus Weevils (“rubble bug, MW) are increasing in importance in the pasture-cropping zone, probably as a result of the shift to reduced tillage systems (Hoffmann et al., 2008). MW are more prevalent on lighter soils, and attack crops (canola, cereals, beans) as well as ryegrass (CESAR, 2008). A current GRDC-funded study (SARDI, 2011) is addressing the biology and control of MW. While results to date suggest that it is not an important pasture pest, it would be prudent to keep a watching brief on MW as a potential pest of pastures in the Mallee areas.

The commercial application of entomopathogenic nematodes for control of weevils and other invertebrate pests is now reasonable well established (Shelton, 2012) but is currently restricted to high value crops. It will be worth assessing whether this approach could become economic for pastures.

8.3.5 Scarabs

Black-headed Cockchafer (Acrossidius tasmaniae, A. pseudotasmaniae, BHC)

These native species are found in south-eastern Australia. The larvae are similar to those of other scarabs but fully grown larvae are smaller than the fully grown larvae of Redheaded and Yellowheaded cockchafer. BHC can be distinguished by the dark colour of their heads as well as their characteristic surface-feeding habit. More mature larvae prefer to attack legumes but will also attack annual grasses. The life cycle of BHC is one year and the later stage larvae cause the greatest damage during winter. BHC can be present in very high numbers. Adult BHC were the most commonly caught scarabs in the summer of 2003-04, in southern NSW, being nearly 90% of all insects caught in that season (Steinbauer and Weir, 2007).

Chemical control can be used because larvae are surface-feeders. Management options to reduce damage caused by BHC include the use of perennial pastures and avoidance of excessive grazing in summer, because the adults prefer to lay eggs in areas with no or low ground cover. While there do not appear to be any commercial biological control options available, diseases and parasitic wasps that attack BHC have been reported (see Pavri and Young, 2007). Early research on biological control options does not appear to have been followed up.

Red-headed Cockchafer (Adoryphorus couloni , RHC)

Red-headed cockchafers are native to eastern and south-eastern Australia. They are difficult to distinguish from some other pasture scarabs. The larvae feed on both annual and perennial grasses as well as subclovers. The RHC has a 2-year life cycle with the final stage larvae causing greatest damage in the second winter. The adults emerge from the soil for only a short period in spring.

Damage to pastures caused by the Red-headed Cockchafer (RHC), sometimes in combination with Yellow-headed Cockchafers, seems to be increasing in severity and incidence in eastern Australia. In 1992, 16,000 ha of improved pasture were severely damaged per annum in Tasmania by these pests (Pauley & Miller 1993). However recent estimates indicate that this figure has grown to 160,000 ha (S. Smith, pers. comm. via J. Shovelton). These scarabs have a broad host range (legume and grasses) and currently there are no chemical control measures available.

As the life cycle occurs almost entirely underground, RHC is a difficult pest to control with chemicals (Heap, 1998; Berg, 2008). Heavy grazing can help to improve badly infested areas because adults like laying eggs in rank pastures, in spring. Annual grasses seem to be more susceptible than perennials, and lucerne does not appear to be susceptible to attack. Ryegrass is very susceptible to RHC, but one particular cultivar, ‘Victoca’, is reported to have some tolerance (Berg, 2008). ‘Victoca’ was selected for tolerance to RHC and is a popular cultivar in the drier parts of Tasmania (Tas Global Seeds, pers. comm.). Cocksfoot and brome can also be grown as RHC-tolerant pasture grasses. Pathogenic organisms such as the fungus *Metarhizium* (*M. anisopliae*) and also birds can reduce the severity of damage caused by RHC, however birds can also cause damage when feeding on larvae. *M. anisopliae* has been used with limited success in first year infestations, but with good results in subsequent years and with good longevity of the fungus in the soil. At present the commercial product (BioGreen™) is not available due to problems with its production.

Yellow-headed Cockchafer (Sericesthis harti, and possibly other species, which used in NSW to include Sericesthis geminata and Sericesthis nigrolineata, YHC)

Larvae of YHC feed on the roots of a wide range of pasture plants. Correct identification of YHC can be difficult. Chemical control of YHC must be done at sowing time. Rotation with crops can help to control this pest, but this option is clearly not available in permanent pastures. While fungal diseases like *Metarhizium* and some predators attack the larvae in the soil, no effective biological controls have been reported for YHC. As with RHC, YHC is a subterranean feeder and there are few effective control measures. Grazing intensity influences populations of these root feeding insects but this has not been used to develop control methods. Chemical control is usually not possible or economic. Resowing pastures with high plant densities and with cultivation prior to resowing are two options.

YHC should be monitored, to determine whether it is an important emerging pest species.

African Black Beetle (Heteronychus arator, ABB)

This introduced pest occurs mainly in Western Australia and along the east coast of Australia. The adult beetles can be confused with RHC and with dung beetles. ABB usually attack grasses (ryegrass, phalaris and others) but not legumes. The greatest damage occurs in summer from later stage larvae, so that perennial pastures rather than annual pastures suffer more significant damage. Ryegrass is often not so badly damaged.

Chemical control of adult ABB in spring can be achieved, but timing is very important (Matthiessen and Learmonth, 1998). Cultural controls include delayed sowing in autumn, and sowing with a legume component and with more tolerant grass species. Fungal endophytes could play a role in protecting grasses from attack by scarabs such as ABB. Fungal endophyte AR542, a non-toxic isolate of *Neotyphodium*, in tall fescue (*Festuca arundinacea*) reduced feeding by adult ABB (Popay et al., 2005).

Corbies (Oncopera species; Lepidoptera)

Oncopera intricata (Corbie) and *O. rufobrunnea* (Winter Corbie) are native species, which are mainly pests of pastures in winter rainfall areas of southeastern Australia. However, other *Oncopera* species (webworms and grassgrubs) can cause substantial damage in the summer rainfall areas of northern NSW and southern Queensland. Corbies have a one-year life cycle. They have a preference for grasses, but can also feed on clovers and other pasture species. Damage to pastures by the larval stages occurs in winter and spring. Thresholds have been established for larval densities that cause severe damage.

Chemical control after grazing can be effective, but depends on timing as a critical factor. Heavy grazing in summer to remove dead plant material can help to control the pest. A number of fungal species have been identified as causing disease in corbies. Ford and Nickson (2004, 2005) trialled two species of entomopathogenic nematode against a Winter Corbie infestation in turf, with very good results from a single treatment applied in spring. There do not seem to have been field evaluations of biocontrol agents in pastures.

8.3.6 Snails and slugs

Small pointed snail (Prietocella barbara), originally from Europe, occurs across southern Australia and is a pest of pastures in higher rainfall areas (> 500 mm). It is a major pest of lucerne in Tasmania and the Bass Strait Islands. Where it is economic to do so, snails can be controlled with chemically-treated bait. A good alternative method of snail control is burning prior to sowing (Micic, 2007).

Slugs. Several species of slug are important agricultural pests in southern Australia. Some species have increased under stubble retention in the pasture-cropping zone (Lawrence, 2009). The two main slug pests of grain crops, which also infest pastures, are the black keeled slug (*Milax gagates*) and the reticulated slug (*Deroceras reticulatum*) which is also known as the grey field slug. Heavy cracking soils favour the development of higher slug populations. Slugs can be controlled with chemically-treated bait pellets if the situation and the economics warrant this approach. Burning of stubble prior to sowing can reduce slug numbers, but does not kills slugs, in contrast to effects of burning on snails (Micic, 2007). Biological control methods for controlling slugs are being actively researched internationally.

8.3.7 Other pasture pests

Black Field Cricket (Teleogryllus commodus)

Black Field Crickets are a major pest on cracking clay soils in winter rainfall areas. Crickets are omnivorous and damage a wide range of pasture species, both legumes and grasses. The most serious damage occurs in late summer and autumn. If levels are over the economic threshold (see Pavri 2007), chemical control with insecticide-treated grain can be effective, especially when little other food is available.

Lucerne Leafroller (Merophyas divulsana)

Lucerne Leafroller is a major, regular pest of lucerne in summer rainfall areas (northern NSW and SE Queensland). It attacks lucerne and clover as well as other crops and weeds. Chemical control does not seem to be an option, and cutting or grazing at the threshold level of the pest is recommended.

Lucerne Leafhopper (Austroasca alfalfae)

Lucerne Leafhopper is a major pest of lucerne in some years, in summer rainfall areas (northern NSW and SE Queensland). Most damage occurs in summer and autumn. Lucerne Leafhopper sucks the sap of the plant, and causes stunting as a result of injecting a toxin. It can also transmit lucerne diseases. Chemical control and grazing management can be used to control this pest.

Native budworm (Helicoverpa punctigera)

Native budworm attacks the above-ground parts of a range of crops and pasture plants, particularly summer legumes. Of the pasture species, Serradella is particularly susceptible to native budworm and lucerne is also susceptible. Native budworm breeds semi-arid inland areas in winter and spring outbreaks are more likely after inland winter rains. Biopesticides such as NPV (Nuclear Polyhedrosis Virus) can be used to treat outbreaks of *Helicoverpa* by spraying eggs and caterpillars. Detailed descriptions of monitoring and control strategies are available (e.g. Brier, 2007; Hertel et al., 2011).

Oxycanus Grass Grub (Oxycanus antipoda)

Oxycanus Grass Grub is a major pest in NW Tasmania and Bass Strait Islands, and a minor pest on the SE Australian mainland and in WA. The life cycle takes 2 years. The larvae feed on grasses but will also feed on clovers when high populations are present. Summer is the main risk period. *Oxycanus* outbreaks seem to be unrelated to the timing of Corbie outbreaks. Little or no research appears to have been undertaken on *Oxycanus* and its control in the past 10 years in Australia.

Pasture Webworms (Hednota species)

Note: some *Oncopera* species (*O. mitocera* and *O. brachyphylla*) are also known as Pasture Webworms; these species are major pests in north Queensland, which is outside the geographic range of this study).

Hednota species are native across southern Australia and attack grasses and cereal crops. Emerging seedlings are particularly at risk. Chemical control (pyrethroids applied to foliage) is effective, but may not be economic on pastures. Cultural control includes heavy grazing before autumn to discourage moths and larvae. There is little information about natural enemies.

8.4 Major pests of different groups of pasture plant species

In this section, the major pests of pasture legumes and grasses are presented, by group of pasture plant. These groups relate to some extent to particular agroecological zones of southern Australia. For example, annual medics are grown in the lower rainfall pasture-cropping zone whereas white clover and other perennial clovers are the basis for permanent pastures in higher rainfall zones.

8.4.1 Scoring system for importance of pests

Detailed information on impact (major / minor), distribution (widespread / local) and timing of occurrence (regular / irregular), for each pest species was taken from chapters in Bailey (2007).

Descriptions:

Major = "Failure to manage likely to result in significant economic loss"

Widespread = "Occurs as a pest .. over most of the range where the [plant] is grown"

Regular = "Likely to be a pest .. in most years"

The rating for a particular pest was in some cases modified slightly after discussion with various experts. A "dot rating" system was developed as follows:

Major, widespread, regular	●●●●●
Major, widespread, irregular	●●●●
Major, local, regular	●●●●
Major, local, irregular	●●●
Minor, widespread, regular	●●●
Minor, widespread, irregular	●●
Minor, local, regular	●●
Minor, local, irregular	●

This information for the main invertebrate pests of the major pasture species is summarized in Table 1. [**NOTE: TABLE 1 is a separate document**].

Table 1 is subdivided by invertebrate pest groupings. Many of the invertebrate pests listed in Table 1 and discussed in the present document were also listed in Table 2 of Panetta et al (1993), which summarized the main arthropod pests of temperate pastures in Australasia. Some are native pests and some have been introduced from other countries. The relative importance of these pests may have changed in the intervening 20 years, but the points made about pest ecology (Panetta et al., 1993) are still highly relevant to our efforts to solve current pest problems.

8.5 Annual Clovers (Trifolium)

8.5.1 Current pest status

8.5.1.1 (A) Research results

This information for annual clovers has been extracted from Table 1.

Major pests in approximate order of importance, considered across the range in southern Australia, for both annual clovers in permanent pastures and in the pasture phases of crop rotations are:

●●●●● Redlegged Earth Mite (RLEM)

●●●●+ Lucerne Flea (almost as important as RLEM)

●●●● Bluegreen Aphid

●●● Note: this “sub-list” is not in order of importance.
Blue Oat Mite, Cowpea Aphid, Small Lucerne Weevil, *Amnemus* Weevil, Sitona Weevil, Black-headed Cockchafer, Red-headed Cockchafer, Black Field Cricket, Corbie, Winter Corbie. Red-headed Cockchafer is regarded by some people as one of the most serious pests in this group, owing to the intractability of the problem (Vic and Tas), but is restricted to some regions.

In annual pastures it is critical to monitor and control pests that attack the seedlings.

There are numerous other pests of annual clovers that are minor in their impact by comparison, although some of these may be locally serious pests. There is disagreement about the importance of Sitona Weevil at present. According to Dennis Hopkins (SARDI, retired; pers. comm., Dec 2011) and James Ridsdill-Smith (CSIRO, retired, pers. comm., Dec 2011), Sitona Weevil is not a major pest of pasture legumes although it may locally be a serious pest. Others say that it should be regarded as a major pest. This situation needs to be resolved.

Clovers that are more resistant to RLEM have been released on the past 2 years by the Department of Agriculture in WA (Nichols, 2010, 2011a, 2011b). For example, the new cultivar

‘Narrikup’ has increased resistance to RLEM both at the seedling (cotyledon) stage and later on in the growth of the plant (Nichols, 2011b).

Yellow-headed Cockchafers are usually not important on pastures and mainly affect cereal crops (P. Umina, pers. comm. Dec 2011). Others are of the view that YHC can be an important pasture pest. This situation needs to be resolved possibly requiring collection of data about the distribution and impact of YHC. The recent survey data (Appendix 2) from Tasmania and the Monaro suggest that this scarab is increasing in importance.

8.5.1.2 (B) Survey Results

There were 41 interviews with consultants. The survey questions are found in the report by Shovelton and Thomas (see Appendix 2). The major concerns, collated by type and frequency of responses to the survey are presented in Table 2.

A semi-quantitative evaluation of the relative importance of pests was carried out.

From the survey of agricultural consultants, the invertebrates currently considered to be the main pests at pasture establishment (in approximate order of importance, see Appendix 2, Figure 5) are: Redlegged Earth Mite, Lucerne Flea, Blue Oat Mite, Black-headed Cockchafer, Bluegreen Aphid, Corbies (*Oncopera* species, especially Tasmania), Snails, Red-headed Cockchafer.

From the survey of agricultural consultants, the invertebrates considered to be the main pests of established annual clovers (in approximate order of importance, see Appendix 2, Figure 9) are: Redlegged Earth Mite, Lucerne Flea, Blue Oat Mite, Corbies (*Oncopera* species, particularly Tasmania), Black-headed Cockchafer, Slugs.

Table 2

Major concerns of agricultural consultants, by type and frequency of response

Region	**Pest**	**Priority**	**Rationale**
General	General	Review economic impact of pests; economic threshold (4)	When to treat problems?
General	General	Pesticide use patterns and non-target effects (5)	Reducing chemical usage
General	General	Information packages on ID and control of pests	
WA, southern NSW	African Black Beetle	Control	No chemicals registered. *[But note that chlorpyrifos and imidacloprid can be used in WA]*

SA	Aphids	Resistance breeding (2) IPM Insecticide resistance status	Cost of insecticides Synthetic pyrethroids are losing effectiveness
SA, WA	Lucerne Flea (LF)	Long term control Understand LF	Currently inadequate control
NSW, SA, Vic, WA	Mites	Insecticide resistance (RLEM) (6) IPM (4) Resistant cultivars (4) Blue Oat Mite (BOM) (3) Bryobia Mite Mites general	What is resistance status in different areas? Alternative to insecticides Against RLEM and BOM (4) Biology & control (3) Biology When to treat? (Timerite?)
NSW, Vic, Tas	Scarabs	Red-headed cockchafer (6) Black-headed cockchafer (3) Corbies (2)	Control / biocontrol & management Control & management Control options
Vic	Slugs		Control of juvenile slugs

8.5.1.3 Gaps in Research and Development

1. Knowledge to enable farmers to reduce pesticide use by applying pesticides more strategically (type, timing, placement, targeting more than one pest if possible). Natural enemies of aphids and mites could play a role (See Investment proposals for Aphids and for Mites).

2. Control of Red-headed Cockchafer (this is an intractable problem in higher rainfall areas).

8.5.1.4 Emerging issues

1. A new biotype of Bluegreen Aphid has appeared, first identified in 2009 (Humphries et al., 2010). This is a potential threat to sub clover pastures and other pasture legumes. It is not yet known whether this new biotype will spread to cooler climates. Cultivar ‘Trikkala’, which is commonly grown, is very sensitive to this new biotype of Bluegreen Aphid.

2. Resistance in RLEM to pyrethroid pesticides (Umina 2007b; Ridsdill-Smith et al., 2008) in WA. There is potential for this resistance to spread regionally and to occur in other states and also potential for pesticide resistance to develop in other invertebrate pests (Ridsdill-Smith et al., 2011).

3. Increased incidence and severity of Balaustium mite (*Balaustium medicagoense*) and Bryobia mite (*Bryobia praetiosa*) (Arthur et al., 2010, 2011).

4. Clover red leaf disease caused by Bean Leaf Roll Virus (BLRV) & its insect vector, the Bluegreen Aphid (Peck et al., 2011). This virus and vector have been identified as occurring in pasture seed production areas. The potential for spread of this disease to pastures more generally needs to be kept in mind. A diagnostic test is available (Peck et al., 2011).

8.5.1.5 Recommendations

1. The new biotype of Bluegreen Aphid, which has overcome plant resistance, is a serious threat to annual clover pastures. Breeding for aphid resistance is in progress at SARDI but not against the new biotype of BGA and only for medic and lucerne – consideration should be given to screening and resistance breeding against BGA in annual clovers. MLA could consider co-investment with the GRDC, and other agencies such as RIRDC, Dairy and Wool RDCs.

2. A study aimed at enabling farmers to reduce pesticide use by applying pesticides more strategically (type, timing, placement, targeting more than one pest if possible; See Investment Proposal on Chemical pesticide use). This study is aimed generally across pasture species. The suggestion is that current commercial usage of insecticides and miticides be surveyed, with a view to rationalising the use of chemical pesticides in pasture systems (amount of chemical used, purpose, timing).

One example: carefully observe the localised distribution of pests and spray only where necessary, e.g. along the side of a paddock where an incursion has been identified. Another aspect could be to look at the movement of pests around a paddock, generating new knowledge that could allow the adoption of a Precision Ag style approach to insecticide use. With reduced pesticide applications, the risks of resistance developing and spreading are decreased.

Natural enemies of aphids and mites could play a role in reducing pesticide use: see Investment proposals for Aphids and for Mites (Ireson and Webb, 1995; Horne et al., 2008; Micic et al., 2008; Roberts et al., 2010).

3. Control of pasture scarabs in high rainfall areas: special attention should be paid to control of Red-headed Cockchafer, which is currently a troublesome pest to control. A research project on this topic could also target the control of Corbies. A project should focus on control methods, as the ecology of these two pests appears to be reasonably well studied (See Investment proposal).

4. Attention should be paid to the potential for Balaustium mites and Bryobia mites to become more widespread and important.

5. Evaluation of the importance of Sitona Weevil and other weevils (e.g. Whitefringed Weevil). This could include screening of Australian cultivars for resistance to Sitona and other weevils.

6. As stated in the General Remarks, there is a strong interaction between grazing management and the control of invertebrate pests and this needs to be taken into account in the design of research programs as well as the application of research results to production systems.

8.6 White Clover (Trifolium repens) and other perennial clovers

8.6.1 Current pest status

8.6.1.1 (A) Research results

As a foreword to this section, it appears that far less information about pest damage and pest control is available for perennial clover pastures (mainly white clover) than for annual clovers. This might be due to a lack of research compared to the annual clovers but could also be due to poor circulation of existing information. A relatively comprehensive document about white clover in Australia is available online through the OGTR (OGTR, 2008). This review contains a section on pests and diseases, however this section of the document is not particularly well referenced.

The information presented here for white clover and other perennial clovers has been taken from Table 1. These legumes are grown mainly in the higher rainfall zones where permanent pastures form the basis of the production system. White clover and other perennial clovers are also grown as irrigated pasture for dairy cattle grazing.

Major pests in approximate order of importance, considered across the range in southern Australia, for perennial clovers in permanent pastures are:

●●●●● Redlegged earth mite (RLEM)

●●●●+ Lucerne flea (almost as important as RLEM)

●●● Note: this "sub-list" is not in order of importance.
Blue Oat Mite, Bluegreen Aphid, Cowpea Aphid, Black-headed Cockchafer, Slugs, Black Field Cricket, Corbie, Winter Corbie & other *Oncopera* species.

There are numerous other pests of white clover that are minor in their impact, although some of these may be locally serious pests. No detail about these pests is presented here, but information can be obtained from sources such as Pavri (2007).

8.6.1.2 (B) Survey Results

From the survey of agricultural consultants, the invertebrates currently considered to be the main pests <u>at pasture establishment</u> (in approximate order of importance, copied from the section on Annual Clovers) are: Redlegged Earth Mite, Lucerne Flea, Blue Oat Mite, Black-headed Cockchafer, Bluegreen Aphid, Corbies (*Oncopera* species, especially Tasmania), Snails, Red-headed Cockchafer.

From the survey of agricultural consultants, the invertebrates currently considered to be the main pests of <u>established perennial clovers</u> (in approximate order of importance, see Appendix 2, Figure 11) are: Redlegged Earth Mite, Lucerne Flea, Blue Oat Mite, Corbies (*Oncopera* species), slugs and snails.

8.6.1.3 Gaps in Research and Development

As indicated above, information about pests of perennial clovers is scarce. As a first step in identifying gaps it will be necessary to collect existing information on major pests and their impact & control into a single review document (an activity that’s not in the scope of this study).

8.6.1.4 Recommendations

1. The area sown to perennial clovers in pastures might be relatively low compared to annual clovers, nevertheless it will be worthwhile to collate existing information on major pests & their control.

2. Some of the recommendations for R&D on annual clovers (e.g. numbers 2. and 6., possibly 3.) also apply to white clovers and other perennial clovers.

8.7 Annual Medics (Medicago spp.)

8.7.1 Current pest status

8.7.1.1 (A) Research results

This information for annual medics has been taken from Table 1, Hopkins et al. (1994) Taverner and Dyson (1993) and Taverner (1995).

Major pests in approximate order of importance, considered across the range in southern Australia, for annual medics which are mainly grown in low to medium rainfall areas (< 500 mm) are:

●●●●● Redlegged Earth Mite (RLEM); note that there is variation between medic species in tolerance to RLEM (PIRSA, 2004).

●●●●+ Lucerne Flea (almost as important as RLEM); note that there is variation between medic species in tolerance to Lucerne Flea (PIRSA, 2004).

●●●● Bluegreen Aphid, Spotted Alfalfa Aphid

●●● Note: this “sub-list” is not in order of importance.
Cowpea Aphid, Sitona Weevil

In annual pastures it is critical to monitor and control pests that attack the seedling stage.

There are numerous other pests of annual medics that are minor in their impact by comparison, although some of these may be locally serious pests. No detail about these pests is presented here, but information can be obtained from sources such as Pavri (2007). As noted in the section on Annual Clovers, there is disagreement about the importance of Sitona Weevil at present.

The pest resistance of different species and also cultivars within species of annual medic can vary widely, depending on the pest. For example, whereas cultivars of Gama Medic are generally moderately resistant to BGA and SAA, cultivars of Burr Medic are variable: some are susceptible to BGA whereas others are moderately resistant. In addition, all cultivars of Burr Medic are susceptible to SAA (PIRSA, 2004).

8.7.1.2 (B) Survey Results

From the survey of agricultural consultants, the invertebrates currently considered to be the main pests of established annual medics (in approximate order of importance, see Appendix 2, Figure 13) are: Redlegged Earth Mite, Lucerne Flea, Bluegreen Aphid, Blue Oat Mite, Snails, Spotted Alfalfa Aphid.

8.7.1.3 Gaps in Research and Development

R&D gaps are similar to those for annual clovers: knowledge to enable farmers to optimise pesticide use and to apply pesticides more strategically (type, timing, placement, targeting >1 pest if possible). Natural enemies of aphids and mites could play a role (See Investment proposals for Aphids and for Mites).

8.7.1.4 Emerging issues

1. As discussed for annual clovers, a new biotype of Bluegreen Aphid has appeared, first identified in 2009 (Humphries et al., 2010). This is a potential threat to medic pastures (and other legumes). There is a current resistance breeding project in progress at SARDI in Adelaide but this does not address resistance to the new biotype of BGA.

2. As mentioned for annual clovers, the development of pesticide resistance in Aphids and in RLEM (Umina 2007b); the potential for pesticide resistance to spread and to occur in other states; and potential for pesticide resistance to develop in other invertebrate pests (Ridsdill-Smith et al., 2011).

3. There are increasing reports of a new weevil (*Mandalotus* spp., or rubble bug) that is becoming a problem for some farmers on lighter soils particularly in minimum tillage/stubble retention systems.

8.7.1.5 Recommendations

1. As for annual clovers, the new biotype of Bluegreen aphid which has overcome plant resistance presents a substantial threat to annual medic pastures. Resistance breeding is in progress at SARDI but does not cover resistance to the new biotype of BGA: it is important to begin screening and breeding for resistance to this new threat. MLA could consider co-investment with the GRDC, RIRDC, Dairy and Wool RDCs.

2. As for annual clovers, a study aimed at enabling farmers to optimise pesticide use and to apply pesticides more strategically (type, timing, placement, targeting >1 pest if possible; See Investment Proposal on Chemical pesticide use). This study is aimed generally across pasture species. The suggestion is that current commercial usage of insecticides / miticides be surveyed, with a view to rationalising the use of chemical pesticides in pasture systems (amount of chemical used, purpose, timing). This proposal suggests that reducing pesticide should be part of a combined set of control measures that targets a range of the most important pests.

3. Research done on the control of RLEM, LF and BGA in medic pastures in the early 1990s led to the development of an "expert system" for decision support on when to treat with pesticides (Hopkins et al., 1994; Taverner 1995). It will be worthwhile to examine this research and to determine whether a simple, up to date web- or mobile phone-based application would be developed for use by graziers.

4. Some of the other recommendations for R&D on annual clovers (e.g. numbers 5. and 6.) also apply to annual medics.

8.8 Lucerne (Medicago sativa)

8.8.1 Current pest status

8.8.1.1 (A) Research results

This information for invertebrate pests of lucerne has been taken from Table 1.

Major pests in approximate order of importance, considered across the range in southern Australia, for lucerne in both permanent pastures and in the pasture phases of crop rotations are:

●●●●● Redlegged Earth Mite (RLEM)

●●●●+ Lucerne Flea (almost as important as RLEM)

●●●● Bluegreen Aphid, Spotted Alfalfa Aphid (form *maculata*), Cowpea Aphid

●●● Note: this "sub-list" is not in order of importance.
Blue Oat Mite, Lucerne Leafhopper, Thrips, Small Lucerne Weevil (especially WA), Sitona Weevil, Small Pointed Snail, Black Field Cricket
For seed producers: the list also includes Etiella (Lucerne seed web moth), Lucerne Leafroller, Lucerne Seed Wasp.

There are numerous other pests of lucerne that are minor in their impact by comparison, although some of these may be locally serious pests and may require R&D to understand and control the pest. No detail about these pests is presented here, but information can be obtained from sources such as Bailey and Goodyer (2007). As noted in the section on Annual Clovers, there is disagreement about the importance of Sitona Weevil at present.

8.8.1.2 (B) Survey Results

From the survey of agricultural consultants, the invertebrates currently considered to be the main pests at lucerne establishment (in approximate order of importance, see Appendix 2, Figure 7) are: Redlegged Earth Mite, Lucerne Flea, Blue Oat Mite, Bluegreen Aphid, African Black Beetle (especially Southern NSW).

From the survey of agricultural consultants, the invertebrates currently considered to be the main pests of established lucerne (in approximate order of importance, see Appendix 2, Figure

15) are: Lucerne Flea, Redlegged Earth Mite, Bluegreen Aphid, White Fringed Weevil, Lucerne Leafroller, Blue Oat Mite, *Heliothis* (*Helicoverpa*).

The responses to questions about research and development priorities yielded the following suggestions (with number of responses in brackets):

Breeding for resistance (7): BOM (1), RLEM (2), Aphids (2), LF (2)

White fringed weevil (6): control measures

Extension (4): provision of more / better material

Insecticides (2): selective insecticides and seed treatments

IPM (1):

Beneficial invertebrates (1)

8.8.1.3 Emerging issues

1. As for annual clovers, a new biotype of Bluegreen Aphid has appeared, first identified in 2009 (Humphries et al., 2010). This is a potential threat to lucerne pastures as well as to other pasture legumes. There is a current Aphid resistance breeding project in progress at SARDI in Adelaide but this does not address resistance to the new biotype of BGA.

2. Whitefringed Weevil could be an emerging threat, judging by the number of comments from consultants about WFW in their research priorities. A recent review of knowledge on WFW (Barnes and de Barro, 2009) and related weevils (Small Lucerne Weevil and Broadback Weevil) advocated research in the following areas:

1. Investigation of aspects of biology: understanding patchy distribution, natural enemies, biological control with nematodes.
2. Further investigation of chemical control options.
3. Alternative cultural control options (other than rotation out of lucerne for up to 3 years).
4. Integration or combination of control measures to maximise chances of success.

8.8.1.4 Recommendations

1. As for annual clovers and medics: screening and breeding for resistance to the new biotype of Bluegreen Aphid.

2. Determine the impact and distribution of Whitefringed Weevil, assess whether it is a serious emerging threat and then, as necessary, evaluate or develop control options.

3. Some of the recommendations for R&D on annual clovers (e.g. numbers 2. and 6., possibly 3. and 4.) also apply to lucerne.

8.9 Other pasture legumes

The main pasture species considered in this category are serradella (*Ornithopus* species), Lotus (*Lotus corniculatus*), Biserrula (*Biserrula pelecinus*), Sulla (*Hedysarum coronarium*) and Sainfoin (*Onibrychis viciifolia*). A recent review of the introduction of new pasture legumes into Australia (Nichols et al., 2008) discusses several of these species in more detail.

At this stage, only information for serradella is included.

8.9.1 Current pest status

8.9.1.1 (A) Research results

General information suggests that serradella species are relatively tolerant of insect pests. The main exceptions appear to be susceptibility to Native Budworm (*Helicoverpa punctigera*) and damage by Lucerne Flea and Redlegged Earth Mite. Chemical control of Native Budworm can be important during pasture establishment (PIRSA, 2005).

8.9.1.2 Gaps in Research and Development

Evaluation of new selections and varieties of the species mentioned above for tolerance or resistance to major invertebrate pests.

8.9.1.3 Recommendations

Some of the recommendations for R&D on annual clovers (e.g. numbers 2. and 6., possibly 3. and 4.) also apply to the newer “other” legumes.

8.10 Sown grasses

8.10.1 Current pest status

8.10.1.1 (A) Research results

The information presented here for sown grasses has been taken from Table 1.

Major pests in approximate order of importance, considered across the range in southern Australia, for sown grasses in pastures are:

●●●● Black-headed Cockchafer (annual grasses), Corbies, Winter Corbies

●●● Note: this “sub-list” is not in order of importance. Redlegged Earth Mite, Red-headed Cockchafer (annual and perennial grasses), Black Field Cricket, *Oxycanus* grass grub, Blue Oat Mite, Webworms (*Hednota*).

8.10.1.2 (B) Survey Results

From the survey of agricultural consultants, the invertebrates currently considered to be the main pests of <u>established grass pastures</u> (in approximate order of importance, see Appendix 2, Figure 17) are: Black-headed Cockchafer, Red-headed Cockchafer, Corbies, Root Aphid, Cutworms, and then African Black Beetle and Black Field Cricket.

8.10.1.3 Gaps in Research and Development

1. While consultants reported that Root Aphid (*Aploneura lentisci)* is an important pest of grassy pastures (especially ryegrasses), there is little published information available in Australia about this pest and Root Aphid was not mentioned in Bailey (2007). Consultants in NSW, Vic and Tas were concerned that there is a lack of information about the impact and distribution of Root Aphid.

2. Control of pasture scarabs in high rainfall areas, especially the problem of controlling Red-headed Cockchafer (see annual clovers).

8.10.1.4 Recommendations

1. Assessment of the importance (i.e. distribution and impact) of Root Aphid on ryegrass pastures in SE Australia. Testing and selection of endophytes of ryegrass could be tried as an approach to controlling the problem.

2. Research on Red-headed Cockchafer and Corbies: as for recommendation 3 under Annual Clovers, research on the control of these beetle and caterpillar pests should include grasses as a target group of plants.

3. Some of the other recommendations for R&D on annual clovers (e.g. numbers 2. and 6., also apply to grasses.

8.11 General comments and observations

* Water use efficiency, pasture productivity and the impact of pests on stock production. Much greater emphasis should be paid to improving our knowledge of these relationships in southern Australian pastures. As an example, a study was recently completed in New Zealand (Morris, 2011) in which the productivity, botanical composition and insect populations present in dryland pasture species were investigated in a long-term grazed field trial. Pasture types included cocksfoot/subterranean clover, cocksfoot/balansa clover, cocksfoot/white clover, cocksfoot/Caucasian clover, ryegrass/white clover and lucerne. Soil moisture and plant water use were measured, and insect pests including Argentine stem weevil, clover root weevil, Sitona weevil and grass grub were monitored. The best pasture compositions, partially based on insect pest tolerance, were identified as a result of this research. Studies of this type would be extremely useful in southern Australian conditions.

* We require a better ecological understanding of some existing pests (Lucerne Flea) and emerging pests (Whitefringed Weevil, Root Aphid, Mandalotus Weevil).

* The impact of pests on flowering and seed set in regenerating pastures needs to be considered, not just the effect on germination and the seedling stage. There can be striking losses in seed set, which have a large impact on regeneration and therefore feed available in subsequent seasons.

* Pesticide resistance.
Depending on where the selection pressure for pesticide resistance is coming from, there may not be a lot that graziers can do. Selection pressures for resistance are certainly coming from

cropping systems (eg pesticide applications to canola) and maybe from pasture legume seed producers, as well as vegetable production areas. However this is no reason for farmers who are managing pastures to be complacent about their own pesticide use patterns: prudence in spray programs is warranted with pastures just as much as in other agricultural production systems.

* The importance of natural enemies of invertebrate pests.
Undoubtedly natural enemies exist in the environment and studies have been made of predatory mites and insects. It is important to find out the extent to which the activity of natural enemies leads to a production benefit. Natural enemies can be encouraged by cultural practices. For example, stubble in minimum tillage systems can be a refuge not only for pests but also for natural enemies. There is a question over whether the populations of natural enemies can build up rapidly enough to exert a significant level of control. An important related question is whether the predatory invertebrates are specialist feeders or generalist, i.e. will they target the pest with sufficient specificity?

* Any new tool to manage pests has to be integrated into the farming system. The combination of several tools can have greater impact. For example the combination of high levels of plant resistance to attack, optimal planting time, managing grazing pressure, chemical controls, enhancing natural enemies and rotation. There are also other methods such as cultivation and burning which might become necessary at times to control certain pests.

* Economic thresholds and production loss data.
As noted in the introduction to this appendix, there is little reliable data available on the economic impacts of pasture pests, and there is almost no recent data (eg last 5 to 10 years). The information currently available was mostly published in the 1990s or even earlier. Nor are the positive impacts of chemical treatments on production well documented.

* Suggestion: could parts of the book *Pests of field crops and pastures: identification and control*, Bailey, P.T. (ed) (2007), be turned into “Ute Guides” with up to date information on pasture pests and their control?

8.12 Acknowledgements

This information was collated from published research and from interviews with researchers as well as a survey of consultants (October – February 2012). The survey population of consultants was subdivided by rainfall and agricultural system, i.e. high rainfall permanent pastures and lower rainfall pastures in crop rotations (see Appendix 2 of this document).

The research information is based on:

(A) Chapters by Pavri (2007), Pavri and Young (2007) and Elder (2007) in Bailey (ed) (2007) as well as recent scientific papers.

(B) Interviews in person or by phone with: Peter Taverner (SARDI), Peter Bailey (SARDI retired), Greg Baker (SARDI), Jake Howie (SARDI), David Peck (SARDI), Alan Humphries (SARDI), Richard Glatz (SARDI), Victor Sadras (SARDI), James Ridsdill-Smith (CSIRO / CRC for Plant Biosecurity, WA), Phil Jobling (CESAR, Melbourne), Paul Umina (CESAR, Melbourne),

Dennis Hopkins (SARDI retired), Mike Grimm (Agriculture WA), Gordon Berg (DPI Vic), Adrian Nicholas (NSW Primary Industries).

8.13 References

Allen, P.G., Lewis, J.G. and Henry, K.R. (1989). *The damage and control of arthropods in medic pasture.* South Australian Department of Agriculture Technical paper No. 25.

Arthur, A.L., Weeks, A.R., Umina, P.A. and Hoffmann, A.A. (2010). (2010) Survival and reproduction of the pest mites *Balaustium medicagoense* and *Bryobia* spp. on winter grain crops. Exp. Appl. Acarol. **52**, 141–153.

Arthur, A.L., Weeks, A.R., Hill, M.P. and Hoffmann, A.A. (2011). The distribution, abundance and life cycle of the pest mites *Balaustium medicagoense* (Prostigmata: Erythraeidae) and *Bryobia* spp. (Prostigmata: Tetranychidae) in Australia. Aust. J. Entomol. **50**, 22–36.

Bailey, P.T. (ed) (2007). *Pests of field crops and pastures: identification and control*. CSIRO Publishing, Melbourne.

Bailey, P.T. and Goodyer, G. (2007). Lucerne. *In* P.T. Bailey (ed.) *Pests of field crops and pastures: identification and control.* CSIRO Publishing, Melbourne, pp 425-439.

Barnes, T. and De Barro, J. (2009). *Improving pest management in the Australian lucerne industry: a review of management of white fringed weevil, small lucerne weevil and broad-back weevil.* RIRDC Publication No. 09/172. RIRDC, Canberra.

Bellotti, W. (1998). Final Report to GRDC; Project UA435. Medic decline syndrome: Problem definition.

Berg, G.N., Williams, P., Bedding, R.A. and Akhurst, R.J. (1993). Biological control of Redheaded Pasture Cockchafer, *Adoryphorus couloni*, using entomopathogenic nematodes. *In* E.S. Del Fosse (ed.). *Pests of pastures: weed, invertebrate and disease pests of Australian sheep pastures.* CSIRO, Melbourne, pp 313–315.

Berg, G. (2008). Biology, importance and management of the redheaded pasture cockchafer *Adoryphorus couloni* (Burmeister). Report to GippsDairy Board Incorporated. http://www.gippsdairy.com.au/Projects/CockchaferTaskforce.aspx

Brier, H. (2007) Summer pulses *in* P.T. Bailey (ed.). *Pests of field crops and pastures: identification and control*. CSIRO Publishing, Melbourne, pp 169-257.

CESAR (2008). PestFacts Southeastern, Issue No 4, Issue No 5. http://www.cesaraustralia.com/sustainable-agriculture/pestfacts-south-eastern/

Edwards, O.R., Franzmann, B., Thackray, D. and Micic, S. (2008). Insecticide resistance and implications for future aphid management in Australian grains and pastures: a review. Aust. J. Exp. Ag. **48**, 1523–1530.

Elder, R.J. (2007). Pastures – summer rainfall. *In* P.T. Bailey (ed.) *Pests of field crops and pastures: identification and control.* CSIRO Publishing, Melbourne, pp 355-369.

Ford, P. and Nickson, D. (2004). Biological control of Winter Corbie (*Oncopera rufobrunnea*) in turf with the entomopathogenic nematode *Heterorhabditis zealandica*. Report for the Victorian Golf Association. http://www.ecogrow.com.au/pdfs/Winter_Corbie_Trial.pdf

Ford, P. and Nickson, D. (2005). Control of Winter Corbie (*Oncopera rufobrunnea* Tindale) in turf with the nematode *Heterorhabditis zealandica* (Strain X1). Proceedings of the 10th International Turfgrass Research Conference, p. 72.

Fulwood, P. (2007). Crop and Pasture Report. PIRSA Rural Solutions. http://www.pir.sa.gov.au/__data/assets/pdf_file/0004/44338/jun07cpr.pdf

Goldson, S.L., McNeill, M.R., Proffitt, J.R. and Barratt, B.I.P. (2005). Host specificity testing and suitability of a European biotype of the braconid parasitoid *Microctonus aethiopoides* as a biological control agent against *Sitona lepidus* (Coleoptera: Curculionidae) in New Zealand. Biocontrol Science and Technology **15**, 791–813.

Grimm, M., Michael, P., Hyder, M. and Doyle, P. (1995). Effects of pasture pest damage and grazing management on efficiency of animal production. Plant Protection Quarterly **10**, 62-64.

Heap, J. (1998). Redheaded pasture cockchafer. PIRSA and the South Australian Research and Development. Institute. Fact Sheet Agdex 430/662.

Hertel, K., Roberts, K. and Bowden, P. (2011). *Insect and mite control in field crops 2011*. NSW Department of Primary Industries.

Hill, M.P., Hoffmann A.A., McColl S.A. and Umina, P.A. (2011). Distribution of cryptic blue oat mite species in Australia: current and future climate conditions. Agricultural and Forest Entomology, DOI: 10.1111/j.1461-9563.2011.00544.x

Hoffmann, A.A. ,Weeks, A.R., Nash, M.A., Mangano, G.P. and Umina, P.A. (2008). The changing status of invertebrate pests and the future of pest management in the Australian grains industry. Aust. J. Exp. Ag. **48**, 1481–1493.

Hopkins, D., Taverner, P., Dyson, C. and Henry, K. (1994). *Cost-effective control of invertebrate pests in annual medic pasture.* Unpublished report to Wool Research and Development Council, Project No. DAS 34.

Horne, P.A., Page, J. and Nicholson, C. (2008). When will integrated pest management strategies be adopted? Example of the development and implementation of integrated pest management strategies in cropping systems in Victoria. Aust. J. Exp. Ag. **48**, 1601–1607.

Howie, J. (1998). The current impact of aphids on medic pastures in the low rainfall alkaline soils zone. Discussion paper for NAPLIP meeting, July 1998, Melbourne.

Humphries, A., Peck, D., Robinson, S., Oldach, K., Glatz, R. and Howie, J. (2010). A new highly virulent bluegreen aphid causes severe damage in previously tolerant pasture and grain legumes. *in* H. Dove and R.A. Culvenor (eds) *Food Security from Sustainable Agriculture*, Proceedings of the 15th Agronomy Society of Australia Conference, available at www.agronomy.org.au

Hyder, M., Thompson, A. and Tanaka, K. (2003). Agronomic implications of intensive grazing systems. Sheep Updates, Department of Agriculture Western Australia. http://www.lifetimewool.com.au/pdf/hyder.pdf

Ireson, J.E. and Webb, W.R. (1995). Effectiveness of *Neomolgus capillatus* (Kramer) (Acarina: Bdellidae) as a predator of *Sminthurus viridis* (L.) (Collembola: Sminthuridae) in northwestern Tasmania. J. Aust. ent. Soc. **34**, 237-240.

James, D.G. (1995). Biological control of earth mites in pasture using endemic natural enemies. Plant Protection Quarterly **10**, 58-59.

Kean, J.M. and Barlow, N.D. (2001). A spatial model for the successful biological control of *Sitona* discoideus by *Microctonus aethiopoides*. J. Appl. Ecol. **38**, 162–169.

Lawrence, L. (2009). Protecting germinating grain crops in southern Australia. Farming Ahead No. 211, pp 49-56.

McDonald, C.L., Nutt, B.J., Devenish, K.L. and Gittins, S.P. (1994). Evaluation of new medic cultivars for the low rainfall wheatbelt of Western Australia: 2. Dry matter availability, liveweight changes and wool production. Proc. Aust. Soc. Anim. Prod., **20**, 229-232.

Matthiessen, J.N. and Learmonth, S.E. (1998). Seasonally contrasting activity of African black beetle, *Heteronychus arator* (Coleoptera: Scarabaeidae): implications for populations, pest status and management. Bull. Entomol. Res. **88**, 443-450.

McQuillan, P., Ireson, J., Hill, L. and Young, C. (2007). *Tasmanian Pasture and Forage Pests: Identification, biology and control.* State of Tasmania. http://www.dpiw.tas.gov.au/inter.nsf/Attachments/LBUN-8KZ3WC/$FILE/Pasture%20Pests%20Book%20final.pdf

Michael, P. J., Grimm, M., Hyder, M.W., Doyle, P.T. and Mangano, G.P. (1997). Effects of pasture pest damage and grazing management on efficiency of animal production. Meat Research Corporation Project DAW.048. Final Report.

Micic, S. (2007). Identification and control of pest slugs and snails for broadacre crops in Western Australia. Department of Agriculture and Food, Western Australia. Bulletin 4713.

Micic, S., Hoffmann, A.A., Strickland, G., Weeks, A.R., Bellati, J., Henry, K., Nash, M.A. and Umina, P.A. (2008). Pests of germinating grain crops in southern Australia: an overview of their biology and management options. Aust. J. Exp. Ag. **48**, 1560–1573.

Morris, N.J. (2011). *Productivity, botanical composition and insect population of seven dryland pasture species in Canterbury after eight years*. M.Sc. Thesis, Lincoln University, New Zealand.

Nair R.M., Robinson, S.S., Haskard, K.A., Dowling. K. and Klingler, J.P. (2007). Identification of resistance to bluegreen aphid and spotted alfalfa aphid in *Trigonella* species. N.Z. J. Agr. Res. **50**, 299-306.

Nichols, P. (2010). Bindoon – a midseason subterranean clover with RLEM resistance. Department of Agriculture, Western Australia. Farmnote No. 420.

Nichols, P. (2011a). Rosabrook – a subterranean clover with increased RLEM resistance for high rainfall pastures. Department of Agriculture, Western Australia. Farmnote No. 454.

Nichols, P. (2011b). Narrikup – a vigorous high rainfall RLEM resistant subterranean clover. Department of Agriculture, Western Australia. Farmnote No. 481.

Nichols, P.G.H., Loi, A., Nutt, B.J., Evans, P.M., Craig, A.D., Pengelly, C., Dear, B.S., Lloyd, D.L., Revell, C.K., Nair, R.M., Ewing, M.A., Howieson, J.G., Auricht, G.A., Howie, J.H., Sandral, G.A., Carr, S.J., de Koning, C.T., Hackney, B.F., Crocker, G.J., Snowball, R., Hughes, S.J., Hall, E.J., Foster, K.J., Skinner, P.W., Barbetti, M.J. and You, M.P. (2008). New annual and short-lived perennial pasture legumes for Australian agriculture—15 years of revolution. Field Crops Research **104**, 10–23.

Nutt, B.J., McDonald, C.L., Devenish, K.L. and Gittins, S.P. (1994). Evaluation of new medic cultivars for the low rainfall wheatbelt of Western Australia: 1. Development of seed reserves over four years under grazing. Proc. Aust. Soc. Anim. Prod. **20**, 225-228.

OGTR (2008). The Biology of *Trifolium repens* L. (White Clover). Version 2. http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/clover-3/$FILE/biologywclover2008.pdf

Panetta, F.D., Ridsdill-Smith, T.J., Barbetti, M.J., Jones, R.A.C. (1992). The ecology of weeds, invertebrate pests and diseases of Australian sheep pastures. *In* E.S. Del Fosse (ed.). *Pests of pastures: weed, invertebrate and disease pests of Australian sheep pastures.* CSIRO, Melbourne, pp 87–114.

Pauley, J. and Miller, C. (1993). Economic evaluation of pasture depredation by corbie and cockchafer larvae. *In* E.S. Del Fosse (ed.) *Pests of pastures: weed, invertebrate and disease pests of Australian sheep pastures.* CSIRO, Melbourne, pp 69–79.

Pavri, C. (2007). Pastures (including lucerne) – winter rainfall. Legume pastures. *In* P.T. Bailey (ed.) *Pests of field crops and pastures: identification and control*. CSIRO Publishing, Melbourne, pp 412–424.

Pavri, C. and Young, J. (2007). Pastures (including lucerne) – winter rainfall. Grass pastures and turf. *In* P.T. Bailey (ed.) *Pests of field crops and pastures: identification and control.* CSIRO Publishing, Melbourne, pp 393–411.

Peck, D., Habili, N., Randles, J., Nair, R., Auricht, G. and de Koning, C. (2011). *Development and Use of Diagnostic Tools for Subterranean Clover Red Leaf Disease.* RIRDC Publication No. 11/107.

PIRSA (2004). *Pasture Legumes for Temperate Farming Systems. The Ute Guide.* PIRSA, Adelaide.

PIRSA (2005). *Serradella*. Fact Sheet No. 62/00. http://www.pir.sa.gov.au/__data/assets/pdf_file/0008/37817/Serradela.pdf

Popay, A.J. and Gerard, P.J. (2007). Cultivar and endophyte effects on a root aphid, *Aploneura lentisci*, in perennial ryegrass. New Zealand Plant Protection **60**, 223-227.

Popay, A.J., Jensen, J.G. and Cooper, B.M. (2005). The effect of non-toxic endophytes in tall fescue on two major insect pests. Proceedings of the New Zealand Grassland Association **67**, 169–173.

Ridsdill-Smith, T.J. (1991). A contribution to assessing the economic impact of redlegged earth mite on agricultural production in Australia. Plant Protection Quarterly **6**, 168-169.

Ridsdill-Smith, T.J. (1997). Biology and control of *Halotydeus destructor* (Tucker) (Acarina: Penthaleidae): a review. Experimental & Applied Acarology **21**, 195-224.

Ridsdill-Smith, T.J., Hoffmann, A.A., Mangano, G.P., Gower, J.M., Pavri, C.C. and Umina, P.A. (2008). Strategies for control of the redlegged earth mite in Australia. Aust. J. Exp. Ag. **48**, 1506–1513.

Ridsdill-Smith, T.J. Sharma, H.C. and Spafford, H. (2011). Sustainable pest management in rainfed farming systems. *In* P. Tow, I Cooper, I. Partridge and C. Birch (eds.) *Rainfed Farming Systems*, Springer Science+Business Media B.V. The Netherlands, pp 253-270.

Roberts, J.M.K., Umina, P.A., Hoffmann, A.A. and Weeks, A.R. (2011a). Population dynamics and diapause response of the springtail pest *Sminthurus viridis* (Collembola: Sminthuridae) in Southeastern Australia. J. Econ. Entomol. **104**, 465-473.

Roberts, J.M.K., Weeks, A.R., Hoffmann, A.A. and Umina, P.A. (2011b). Does *Bdellodes lapidaria* (Acari: Bdellidae) have a role in biological control of the springtail pest, *Sminthurus viridis* (Collembola: Sminthuridae) in south-eastern Australia? Biological Control **58**, 222–229.

Roberts, R.J. and Ridsdill-Smith, T.J. (1979). Assessing pasture damage and losses in animal production caused by pasture insects. *In* T.K. Crosby and R.P. Pottinger (eds) Proceedings of the 2nd Australasian Conference on Grassland Invertebrate Ecology. Government Printer, Wellington NZ, pp 124-125.

Roberts, R.J., and Morton, R. (1985). Biomass of larval Scarabaeidae (Coleoptera) in relation to grazing pressures in temperate, sown pastures. J. Appl. Ecol. **22**, 863-874.

Rose, R. (2008). *Medicago truncatula* as a model for understanding plant interactions with other organisms, plant development and stress biology: past, present and future. Functional Plant Biology **35**, 253-264.

SARDI (2011) Pest Facts Issue No 1. http://www.sardi.sa.gov.au/__data/assets/pdf_file/0010/154675/PestFacts_Issue_1_2011.pdf

Sexton, S.B. and Williams, P. (1981). A natural occurrence of parasitism of *Graphognathus leucoloma* (Boheman) by the nematode *Heterorhabditis* sp. J. Aust. Entomol. Soc. **20**,

Shelton, A. (2012). Biological control. A guide to natural enemies in North America. http://www.biocontrol.entomology.cornell.edu/pathogens/nematodes.html

Sloane, Cook and King Pty Ltd (1988) The economic impact of pasture weeds, pests and diseases on the Australian wool industry, Australian Wool Corporation, Melbourne.

Steinbauer, M.J. and Weir, T.A. (2007). Summer activity patterns of nocturnal Scarabaeoidea (Coleoptera) of the southern tablelands of New South Wales. Aust. J. Entomol. **46**, 7–16

Taverner, P.D. and Dyson, C.B (1993). Invertebrate pests associated with annual medic pastures in South Australia. *In* Proceedings of the 7th Australian Agronomy Society Conference. http://www.regional.org.au/au/asa/1993/poster/germination-establishment/1993pgs342-poster2c-07.htm

Taverner, P. (1995). How to win the war against medic pests *in* M. Casey (ed.) *Pasture plus: the complete guide to pastures*. Kondinin Group, Belmont WA, pp. 345 – 346.

Umina, P. (2007a). Redlegged Earth Mite. Victorian Department of Primary Industries Note AG0414. http://www.dpi.vic.gov.au/agriculture/pests-diseases-and-weeds/pest-insects/redlegged-earth-mite

Umina, P. (2007b). Pyrethroid resistance discovered in a major agricultural pest in southern Australia: the redlegged earth mite *Halotydeus destructor* (Acari: Penthaleidae). Pest Management Science **63**, 1185–1190.

Young, J.M., Ridsdill-Smith, T.J., Gillespie, D.J. and Michael, P.J. (1995). The value of reducing redlegged earth mite damage in pastures and the impact on optimal farm strategies for the great southern region of Western Australia. Plant Protection Quarterly **10**, 67 – 68.

Table 6 Major Invertebrate pests of pastures in Southern Australia based on published literature and interviews with researchers

	Annual clovers **Subterranean** **Balansa** **Persian** **Arrowleaf** **Rose** **Gland, etc**	**Perennial clovers** ***(Trifolium)*** **White(*T. repens*)** **Strawberry (*T fragiferum)*** **Red (*T. pratense*)** **Caucasian (*T. ambiguum*)**	**Annual medics** **(*Medicago* spp)** **Strand** **Barrel** **Burr** **Murex** **Snail**	**Lucerne** **(*Medicago sativa*)**	**Other legumes** **Serradella** **Lotus** **Biserrula** **Sulla** **Sainfoin**		**Grasses** **Phalaris** **Cocksfoot** **Ryegrass (annual & perennial)** **Tall fescue** **Puccinellia**	**NOTES**
Mites								
Redlegged Earth Mite (I)*Halotydeus destructor*	●●●●●	●●●●●	●●●●●	●●●●●	●●●		●●●	**Major, widespread, regular (temperate)**
Blue Oat Mite (I) *Penthaleus* spp.	**?**	**?**	**?**	●●●	●●●		●●●●●	
Springtails								
Lucerne Flea (I) *Sminthurus viridis*	●●●●●	●●●●●	●●●●●	●●●●● **?**	●●●●●		●● **(Ryegrasses)**	**Major, widespread, regular**
Aphids								
Bluegreen (BGA) (I) *Acyrthosiphon kondoi*	●●●●	●●●●	●●●●	●●●●	●●●		**?**	**Major, widespread, regular** **SAA 2 biotypes: one vs lucerne, other vs clover**
Spotted Alfalfa (SAA) (I) *Therioaphis trifolii*	●●	●●	●●	●●●●	●●		**?**	
Cowpea (CPA) *Aphis craccivora*	●●●	●●●	●●●	●	●●●		**?**	

Pea (PA) *Acyrthosiphon pisum*	●●	●●	●●	●●●●	?		?	
Leafhoppers								
Lucerne Leafhopper *Austroacsa alfalfa*				●●●				**NSW, Qld: Major, irregular**
Thrips								
Onion *Thrips tabaci* Plague *T imaginis* Tomato *Frankliniella schultze*				●●●				**Major, widespread, regular**
Weevils								
Sitona (I) *Sitona discoideus*			●	●				**Minor SA NSW, Tas, Vic (D Hopkins pers. comm.)**
Small Lucerne Weevil *Atrichonotus taeniatulus*	●●●			●●●				**WA (major, restricted, regular) / NSW**
Amnemus Weevils *Amnemus* spp.	●●●●							**Nth NSW, SE Qld: widespread, frequent**
Whitefringed Weevil (I) *Naupactus leucoloma*				●●				**Minor widespread irregular (lucerne)**
	Annual clovers	**Perennial clovers**	**Annual medics**	**Lucerne**	**Other legumes**		**Grasses**	**NOTES**
Scarab Beetles								

Blackheaded Cockchafer (I) *Acrossidius* (= *Aphodius*) spp.	●●●	●●●	**?**	**?**	**?**		●●●● **(annual)**	**Southern areas; older larvae prefer legumes**
Redheaded Cockchafer *Adoryphorus couloni*	●●●			**Not attacked**			●●● **(annual + perennial)**	**NSW, Qld, SE of SA, Vic, Tas; major restricted, irregular INTRACTABLE**
African Black Beetle								**WA only**
Snails & slugs (I)								
Small Pointed Snail *Prietocella barbara*				●●●				**Major, restricted, regular (Tas)**
Conical Snail *Cochlicella acuta*								
Slugs		●●●						
Crickets								
Black Field Cricket(I) *Teleogryllus commodus*	●●●	●●●	**?**	●●●	**?**		●●●	**Winter rainfall areas, cracking clays (major, restricted, regular)**
Caterpillars								
Corbies *Oncopera intricata*	●●●	●●●					●●●●	**Tas widespread, irregular**
Corbies (I) *Oncopera spp*	●●●	●●●					●●●●	**Sth NSW, wetter Tas, widespread, irregular**
Oxycanus Grass Grub *Oxycanus antipoda*	●	●					●●● / ●	**NW Tas / SE Aust & SW WA**

Etiella (Lucerne seed web moth) *Etiella behri*				•••				**Sth mainland major, irregular (seed crops)**
Lucerne Leafroller *Merophyas divulsana*	• **?**	• **?**		••••				**NSW / Qld; Major, regular**
Seed wasp								
Lucerne Seed Wasp *Bruchophagus roddi*				••••				**Major, widespread (greater in south), irregular; (seed crops)**
Native Budworm								
Helicoverpa punctigera	**?**	**?**	**?**	••	•••		**?**	**Serradella seed crops affected**
Pasture Webworm (I) *Hednota* spp							•	
Brown Pasture Looper (I) *Ciampa arietaria*	•	•						

The migratory locust, wingless grasshopper and Australian plague locust were not considered in this review

INVERTEBRATE PESTS SCORING

Major, widespread, regular	●●●●●
Major, widespread, irregular	●●●●
Major, local, regular	●●●●
Major, local, irregular	●●●
Minor, widespread, regular	●●●
Minor, widespread, irregular	●●
Minor, local, regular	●●
Minor, local, irregular	●

9. Appendix 5

Barbetti & Jones – Roles of fungal, viral and nematode infections in causing decline pasture health across Australia

9.1 Overall summary of gaps and research needed to address decline in pasture health across southern Australia

9.1.1 Critical gaps and research needed

- Define the pathogen strain/race structures for *Phytophthora, Kabatiella* and three major virus pathogens (SCMoV, BYMV and AMV) across southern Australia, and determine associated host resistances against specific strains and races of these pathogens such that available host resistances can be strategically and effectively deployed.
- For major fungal and viral pasture diseases, define relative resistances and/or tolerances of all near-release breeding genotypes across all major pasture species to other major fungal (*Pythium, Cercospora*, rust, powdery mildew, *Aphanomyces*), and viral (SCRLV, WCMV, BYDV and RyMV) pathogens, and identify sources of resistance to these pathogens for breeders to utilise.
- Surveys to determine incidence and impacts (losses and economic importance) of major fungal and viral diseases in lucerne, alternative annual and perennial pasture legumes, and grasses across southern Australia.
- Identify and demonstrate the benefits to farmers from cultural practices (e.g., grazing, cultivation) for reducing the severity and impact of major fungal and viral diseases in pastures across southern Australia. Establish best practices for ensuring seed stocks of new variety releases of all pasture legumes are free of seed-borne virus infection.
- Identify cost-effective chemical seed treatments for effective control of pre- and post-emergence damping-off of pasture legumes when re-sowing pastures and for controlling virus spread by aphid and mite vectors.

9.1.2 Training and succession planning

Clarke and Eagling (1994), Jones (1996), Barbetti and Jones (1996) and Barbetti et al. (2005) all reported an alarming trend towards the reduction of pasture plant pathology expertise throughout Australasia. They also emphasised that this trend needs to be reversed to allow adequate resourcing of diagnostic capabilities, disease management and breeding programmes to manage current plant health challenges from fungal, oomycete, viral and nematode disorders and also to ensure the ability to effectively respond to any new threats arising, including the introduction of any exotic pathogens (e.g., WSMV in grasses) because the relevant researchers were close to retirement age. This trend has not altered in any way following these reviews, except that the expertise across Australia has now largely evaporated!

There is urgent need to train up the next generation of pathologists (particularly for fungal, oomycete and viral pathogens) for southern Australia and currently only Western Australia has this capacity but because the relevant researchers are at retirement age, this opportunity will be lost to transfer these skills to the next generation if action is not taken quickly.

9.1.3 Introduction

Maintaining pasture health remains an ongoing and significant challenge for farmers. This is partly because of the high cost of intervention strategies and partly because farmers and their advisers have difficulty in recognising the symptoms of most fungal, oomycete, viral and nematode diseases in pastures. This is especially so where they occur in mixed species swards often with a significant component of weeds. The problem of lack of recognition of pasture disorders particularly relates to foliar disease symptoms caused by viruses where foliage symptoms are subtle and by root pathogens and nematodes occurring below ground and largely out of sight. This recognition problem is compounded by lack of knowledgeable pasture advisers and consultants in a position to provide relevant advice to farmers and by a severe shortage of suitably trained scientists in all aspects pasture pathology in Australia.

Pastures are planted with one or more of a range of annual or perennial legume and grass species Over
400 fungal, bacterial, viral, phytoplasmal and nematode pathogens cause pasture diseases that impair productivity of Australian pastures (Haggar *et al*. 1984; Johnstone and Barbetti 1987; Buchen-Osmond et al. 1988; Panetta et al. 1993; Johnstone and McLean 1987; Barbetti et al 1996; Jones 1996, 2004; Murray and Davis 1996) and thereby adversely affect the profitability of grains, dairy meat and wool industries across southern Australia, but in particular our meat industries (Barbetti et al. 2005).

While the ranges of pathogens that cause diseases in different annual and perennial pasture species vary, many are common to a number of species or even across different genera of annual and perennial pasture legumes, or within different grass species (Johnstone and Barbetti 1987: Panetta et al. 1993). The textbook 'Viruses of Plants in Australia' by Buchen-Osmond et al. (1988) lists all viral pathogens then known to infect annual and perennial pasture legume and grass species. Virus diseases of annual and perennial pasture grasses and legumes were reviewed by Jones (1996). Earlier general reviews (Johnstone and Barbetti 1987; Panetta et al. 1993) provided only limited coverage of pathogens of lucerne, or annual or perennial grasses, and did not cover perennial pasture legumes other than lucerne, nor newer alternative *Trifolium* spp. or other annual pasture legume genera. There were also earlier more specific reviews about fungal and viral diseases of grasses (Clarke and Eagling 1994), foliar fungal pathogens (e.g., Barbetti and Sivasithamparam 1986) and viral diseases of subterranean clover (Johnstone and MacLean 1986), or white clover (Johnstone and Chu 1993). Annual medic pastures were reviewed recently in relation to both root and foliar fungal diseases (Barbetti et al. 2006). For subterranean clover, they were reviewed within the decade since year 2000 in relation to root disorders (Barbetti et al. 1986; Barbetti et al. 2007). Seed-borne viral diseases of subterranean clover and annual medic were major components of other reviews in the same decade (Jones 2000, 2004a). However, fungal diseases of perennial clovers and viral diseases of lucerne and recent, alternative pasture species have never been reviewed comprehensively.

Pathogens cause losses that adversely affect animal feed availability largely by reducing herbage and seed yields. For subterranean clover and annual medics, the losses they cause were reviewed to varying extents previously for Australia (Johnstone and Barbetti 1987; Johnstone and Mclean 1987; Panetta et al. 1993; Pottinger et al. 1993; Barbetti and Jones 1999; Barbetti et al. 1996b; Jones 1996, 2000, 2004a; Barbetti et al. 2005, 2006, 2007). While seed and herbage yield losses caused by one or more individual fungal and viral pathogens in grazed or ungrazed swards were well quantified historically in subterranean clover (Barbetti 1987c; Barbetti and Nichols 1991a; Barbetti 1996; Wroth and Jones 1992; Jones, 1991, 1992, 1994; Ferris and Jones 1995), losses occurring in annual medics have only been quantified well for viruses in burr medic (*Medicago polymorpha)* (Jones and Nicolas 1992, 1998).

The latter study included the demonstration that presence of a viral pathogen causing mild foliar symptoms in burr medic still greatly influenced its ability to compete with weed or grass species resulting in major changes in the species composition of grazed swards and causing predominance of weeds (Jones and Nicolas 1998). The impacts of pathogens on lucerne, white clover, and ‘alternative’ or ‘newer’ pasture legumes such as Balansa clover and Serradella, have never been quantified in pasture swards in the field in Australia. Also, the only quantification of losses in grass swards has occurred with viruses and these studies were rather limited (Eagling et al. 1989b, 1992). Importantly, the indirect losses from pathogens including diminished host persistence, residual fixed nitrogen, utilisation of inputs to plant growth (*e.g*., inefficient fertiliser and water use), and animal productivity and, also, increased cost and side-effects of control (Barbetti et al. 1996b) have been largely ignored. It is noteworthy that before 1970, diseases of pastures were erroneously considered not to be a significant problem in Australia and therefore not to warrant control measures (Anon. 1988). Perhaps the only significant ‘contribution’ of that particular report was to largely negate both the funding and research into pasture health for the subsequent three and a half decades. This still occurred despite the many reports of declining production across Australian medic and/or subterranean clover pastures resulting from disease (e.g., Bellotti and Kerby, 1993; Barbetti et al. 1996b).

This report:-

(i) outlines the major and/or most widespread disorders caused by fungal, oomycete, viral and nematode pathogens in pastures, including those infecting pasture species not reviewed previously;

(ii) emphasises the roles of a spectrum of pasture fungal, oomycete, viral and nematode pathogens in causing productivity losses;

(iii) highlights recent changes in pathogen disorders, particularly in relation diseases of ‘alternative’pasture legumes as these were have never been reviewed;

(iv) focuses on foliar fungal disorders of subterranean clover which have not been reviewed comprehensively for 25 years;

(v) summarizes the spectrum of control options for management; and

(vi) highlights areas of research deficiency limiting opportunities for maintaining or improving pasture health and productivity for the Australian meat industry.

This report does not review areas relating to diminished health of the grazing animal as a consequence of toxicity or biological organism associations with particular pasture components, such as annual ryegrass toxicity, flood plain staggers, ergot on grasses such as paspalum and ryegrass, and kikuyu poisoning. In the same way, phytoestrogen disorders in pasture legumes, either consequent from pathogen involvement (e.g., Croker et al. 1994a,b, 1999, 2005; Jones and Ferris 2001), or unrelated to presence of pathogens, are not included.

Although viral diseases are strictly speaking not “foliar diseases’ because viruses invade the whole plant including the roots where they also cause damage, for simplicities sake they are included here under ‘foliar diseases’ because their symptoms are normally most evident in the above ground portions of plants.

9.2 Annual clovers

9.2.1 Soil-borne diseases of annual clovers

9.2.1.1 Introduction

Fungal and nematode soil-borne diseases of annual clovers were comprehensively reviewed recently by Barbetti et al. (2005), and for subterranean clover only by Barbetti et al. (2007). However, a summary of key information in relation to soil-borne diseases of clovers is given below to provide all relevant information in this one report, along with additional information that became available afterwards. The majority of soil-borne diseases that occur on subterranean clover in general also occur across other annual *Trifolium* species such as Persian, Purple, and Balansa and research relating to these species is reviewed for the first time here.

9.2.1.2 Disease Symptoms

Root rots of subterranean clover are widespread across southern, western and eastern Australia. The above ground symptoms of root rots in subterranean clover vary in different localities, possibly because of environmental differences (Barbetti and MacNish 1983) and also nutritional influences. Typical symptoms include stunted yellow-green, yellow-red, or red-purple plants (Burgess et al. 1973; Barbetti and MacNish 1983; Clarke 1983b) scattered among apparently healthy plants, or the affected areas may occur in distinct patches (Barbetti and MacNish 1983; Clarke 1983b). In some situations, big areas of subterranean clover within a paddock may be diseased (Barbetti and MacNish 1983; Clarke 1983b), in others whole paddocks may be affected (Barbetti and MacNish 1983).

<u>Victoria</u>*:* In Victoria, Clarke (1983b) described the root symptoms as tap roots with a dark brown to black wound about 10-20 mm below the soil surface. These wounds are about 5-10 mm in length, girdle the root, often severing the tap root at the wound, so leaving the plant on the soil surface without functioning roots. Burgess et al. (1973) reported that root systems are rotted to varying degrees with brown to black lesions at the junction of the lateral and tap root, along the lateral roots, and at the root tip. They reported the frequent occurrence of a marked reddish-brown discolouration of the stele of affected lateral and tap roots. This discolouration frequently extended into the crown and affected root systems were often completely necrotic.

New South Wales: In NSW, Stovold (1971) reported extensive rotting of the lateral feeder roots and stunted roots on affected plants.

Western Australia: In Western Australia, Barbetti and MacNish (1983) noted root symptoms involving part or all of the root system with the tap root usually being more damaged than the laterals. The internal and external tissues of diseased roots are brown and discoloured. MacNish *et al*. (1976) also reported a rot of the tap root confined to 10 to 20 mm below the crown in subterranean clover in irrigation areas. Sometimes affected plants can produce new lateral roots above the lesions on the tap root, and these plants may slowly recover (Barbetti and MacNish 1983; Clarke 1983b).

9.2.1.3 Scope, extent and impact of soil-borne disease on pasture productivity

Root rot has seriously adversely affected the production from subterranean clover pastures over at least the past 4 decades but most likely much longer (Johnstone and Barbetti 1986; Barbetti et al. 2005, 2007).

Victoria: In Victoria, the problem was first recognized in 1960 (Anon. 1960) and is a serious problem which reduces the productivity of pastures (Taylor 1984, 1985; Taylor et al. 1984). A survey of subterranean clover pastures in 1970 (McGee and Kellock 1974) showed that root rots were widespread in northern, southern and eastern Victoria, with more than 70% of plants in some pastures showing root rot symptoms. In some pastures up to 90% of plants in a stand were affected by the disease (Clarke 1983b). A subsequent study by Burnett et al. (1994) showed that decline in permanent pastures in north- eastern Victoria remains an on-going problem. Greenhalgh and Clarke (1985) used drenches of fungicides metalaxyl (against *Pythium* and *Phytophthora*) and benomyl (against *Fusarium*) in 1981 to study significance and etiology of root rot of subterranean clover in dryland subterranean clover pastures in Victoria. They examined sites located at Clunes in Western Victoria north of Ballarat and demonstrated significant increases in subterranean clover herbage production following fungicide application. Taylor et al. (1983) investigated root rot of irrigated subterranean clover in northern Victoria, both its significance and the prospects of control. These studies demonstrated increases of nearly 60% in May and more than 95% in October in herbage production from where the fungicide applications were made.

NSW: In N.S.W., Valder (1954) reported that root rot disease was usually not important except in stands of red clover. A problem of poor re-establishment and poor forage and seed production in long-term subterranean clover pastures has been recognized since the mid 1960's (Anon. 1965, 1968, 1969, 1970, 1971). Investigations by Stovold (1974a) showed that root rots were an important factor in this observed decline of established subterranean clover pastures. Hochman et al. (1990) examined the factors contributing to reduced productivity of subterranean clover pastures on acid soils at four field sites with poor clover growth (Holbrook, Yerong Ck, Oberne, Lankey's Ck) in NSW and utilized multiple treatments including P, Mo, lime pelleted seed, lime additions, metalaxyl (against *Pythium* and *Phytophthora*), ryegrass competition, lime+Mg+trace elements. They demonstrated increases in herbage yields of the order of 10-12% from these treatments.

Western Australia: In Western Australia, decline was first recognised by Shipton (1967b). Large areas in the south-west and south coastal districts have been affected by pasture decline due to root rot (MacNish et al. 1976; Gillespie 1983c), with heavy production losses resulting from severe root rot in situations where a big percentage of seedlings are killed even prior to emergence and where emerged seedlings die from root rot in the first few weeks of the growing season (Barbetti and MacNish 1983). Wong et al. (1985b) demonstrated that seedling losses in the field from damping-off could exceed 90%. Barbetti (1984e) showed an inverse relationship between the severity of rotting of the tap root system and plant size. The greatest reduction in plant size from root rot occurred from 6 or 7 weeks after emergence till 16 or 17 weeks into the growing season. Such reductions often exceeded 70% suggesting that production from pastures with severe tap root rot may be very poor. Wong et al. (1986a) described the inverse relationships between plant size and root disease for a range of soil-borne pathogens. Recently, O'Rourke et al (2009) demonstrated that it was not just root disease on seedling and young annual clover plants that was important, but that root disease on mature subterranean clover plants can also be severe and significantly reduce productivity of annual pasture legumes.

<u>Across southern Australia:</u> More recently, Simpson et al. (2011) used field-based plant bioassays to assess the potential for pre- and post-emergence loss of seedlings and for root damage affecting subterranean clover during autumn-winter at 17 locations across a broad agricultural area of temperate southern Australia. Between 9 and 93% (median 21%) of *T. subterranean* seedlings failed to emerge at the 14 locations where soil moisture was considered adequate for germination, Post-emergence losses were lower (range 0-32%; median 7%). Moderate (lateral roots) to severe damage (tap roots) was recorded on surviving test plants at all of the sites. These studies indicated that sub-lethal damage to pasture roots constitutes a potentially large, but underestimated cost to production because it was so widespread and because the damage occurs during autumn-winter when pasture yield limits stocking rate. DNA assays for common root rot disease pathogens (*Pythium irregulare*, *Phytophthora clandestina*, *Rhizoctonia*) were used for the first time to construct cost-effective profiles of fungal and oomycete pathogens at each site. Such assays may be useful for indicating disease risks, guiding plant variety selection and appropriate use of pesticides.

9.2.1.4 . Fungal and oomycete pathogens involved

<u>Victoria:</u> In Victoria, a number of soil-borne fungi have been shown to be associated with root rot. In the first record of root disease (Anon. 1960) it was reported that *Fusarium* spp. and *Rhizoctonia* spp. were consistently isolated from infected plants. Kellock (1972) and McGee and Kellock (1974) demonstrated with pathogenicity tests that *Fusarium avenaceum* was highly pathogenic while *F. oxysporum* was only weakly pathogenic. In subsequent tests (Kellock et al. 1978) further *F. avenaceum* isolates were shown to be highly pathogenic while *F. oxysporum* showed no effect either alone or in combination with *F. avenaceum*. Kollmorgen (1974) demonstrated that *F. avenaceum* reduced emergence and dry weight of subterranean clover. Studies by Burgess *et al*. (1973) showed that *F. roseum* "'Avenaceum", *F. roseum* 'Sambucinum" and *Pythium irregulare* were highly pathogenic while *F. oxysporum*, *F. culmorum* and probably *F. avenaceum*, although commonly associated with diseased roots, were weakly pathogenic. Greenhalgh and Lucas (1984) consistently isolated *P. irregulare*, *P. mamillatum* and *F. avenaceum* from necrotic roots and all were demonstrated to be highly pathogenic in sand but only slightly pathogenic in untreated soil suggesting that the presence of other organisms interferes with their pathogenicity. Also in Victoria, Smiley et al. (1986), using intact field cores, identified that a complex of pathogens, including species of *Pythium, Fusarium, Rhizoctonia*, and nematodes, were involved in root disease of subterranean clover in that state. That a complex of pathogens was involved in root disease of subterranea n clover was reaffirmed in a review of this issue undertaken by Flett and Clarke (1996). In 1982 a new *Phytophthora* sp. was detected on rotted taproots of subterranean clover in Victoria, its pathogenicity confirmed (Taylor 1984; Greenhalgh and

Taylor 1985; Taylor et al. 1985c), and was subsequently described as *Phytophthora clandestina* (Taylor et al. 1985c). Greenhalgh (1992) estimated that the disease caused by this pathogen could reduce annual production of subterranean clover by more than 90%. Severe disease particularly occurred in long growing seasons and in the irrigated areas in Victoria (Taylor *et al.,* 1985b; Greenhalgh and Flett, 1987; Taylor and Greenhalgh, 1987). Studies with *P. clandestina* in the 1980's assumed the existence of only a single race of *P. clandestina* in Victoria. However, a later study by Flett (1994) of ten *P. clandestina* isolates on 5 subterranean clover varieties, showed that varieties Larisa and Trikkala, which previously had been classified as resistant, were susceptible to 2 isolates. They designated earlier isolates as race 0 while the 2 latter isolates were designated as race 1 (Flett 1994). Subsequently, Purwantara et al. (1998, 2001) identified 3 additional races (*viz*. races 2, 3, and 4) using four to six varieties and up to 112 isolates of *P. clandestina* collected from different geographic regions across southern Australia. In 1985, Greenhalgh et al. (1985) reported *Aphanomyces euteiches* associated with subterranean clover root rot, demonstrated its pathogenicity, and suggested that it was an important pathogen in both northern and southern Victoria.

NSW: In N.S.W., investigations by Stovold (1971, 1974a,b) showed that *Pythium* spp., in particular *P. irregulare*, were the most common fungi isolated from diseased roots. Pathogenicity tests demonstrated that *P. irregulare* consistently caused damping-off of germinating subterranean clover. *Pythium* species were also associated with a white clover decline problem on the north coast of NSW (Stovold and Wong, 1973). *P. clandestina* was shown to be an important root pathogen of subterranean clover in NSW by Dear et al. (1993).

Western Australia: In Western Australia, Shipton (1967b) showed that *F. oxysporum* and *F. avenaceum* were the most frequently isolated fungi from diseased roots. He found that *F. oxysporum*, *F. graminearum*, and *F. moniliforme* were highly pathogenic under non-competitive conditions. Barbetti and MacNish (1978) isolated *P. irregulare*, *P. debaryanum*, *P. acanthicum*, *P. middletonii*, *F. oxysporum*, and *Rhizoctonia* spp. from root rot-affected subterranean clover in the irrigation areas of south-western Western Australia. They showed that the three most frequently isolated fungi; *viz. P. irregulare* in particular, *P. acanthicum*, and *F. oxysporum* could cause root rot and reduce seedling emergence, particularly following inoculation with two or more fungi in combination in comparison with application of a single fungus. Wong et al. (1984) showed that *F. avenaceum*, *P. irregulare*, and *R. solani* were highly pathogenic while *F. oxysporum* and *Phoma medicaginis*, particularly when tested singly, were only weakly pathogenic. However, Wong et al. (1986e) showed that isolates of *F. oxysporum* vary in pathogenicity. Compared with individual fungi, mixed fungal inoculum increased the severity of root disease and decreased plant survival and plant weight. Wong and Sivasithamparam (1985) determined the association of *Rhizoctonia* and *Waitea* spp. with root rot of subterranean clover in Western Australia, and subsequent pathogenicity tests (Wong et al. 1985a) showed that *R. solani* anastomosis (hyphal fusion) groups 2-1 and 2-2 were highly virulent, *R. cerealis*, anastomosis group F and unassigned isolates varied in virulence, anastomosis group K isolates were non-pathogenic and that a *Waitea* sp. caused only mild damage to tap roots. Studies conducted by Wong et al. (1985b) into the identity and pathogenicity of fungi associated with root rot of subterranean clover in Western Australia showed *P. irregulare* and *F. oxysporum* to be the most frequently isolated fungi. *F. avenaceum*, *P. irregulare*, *P. spinosum*, and *R. solani* were highly pathogenic to subterranean clover seedlings. *F. oxysporum* and *P. medicaginis* were less pathogenic and *F. acuminatum*, *F. culmorum*, *F. equiseti*, one isolate of *M. phaseoli*, and *Waitea circinata* were only weakly pathogenic. *Ceratobasidium* sp. *F. sulphureum*, one isolate of *M. phaseoli*, *P. coloratum*, and *R. cereale* were non-pathogenic. *P. clandestina* was also frequently detected. More recently, Barbetti (2005) demonstrated that *Cylindrocarpon didymum* also infected and damaged tap and lateral roots of subterranean clover and reduced plant dry weights. He also demonstrated that *C. didymum* produced brefeldin A *in vitro* and the frequently observed stunted appearance of affected tap and lateral roots was possibly as a consequence of production of brefeldin A in the root tissue by this pathogen.

Western Australia – *Phytophthora clandestina:* In Western Australia, the presence of *P. clandestina* was confirmed in 1984 (Taylor et al. 1985a). Wong et al. (1984) showed that *P. clandestina* interacted with *F. oxysporum*, but not with *F. avenaceum*, *Phoma medicaginis*, *Pythium irregulare*, or *R. solani*, to produce more severe root rot than did either fungus alone. Further, Wong et al. (1986c) described the interactions of *P. clandestina* with soil temperature and moisture; Wong et al. (1986b,d) defined the soil behaviour of this pathogen, including in the field; while Wong et al (1986e) defined the influence of environmental factors on the growth and survival of *P. clandestina*. Barbetti (1989d) reported that the most susceptible varieties to Western Australian isolates of *P. clandestina* were Woogenellup, Green Range, Mt Barker and Esperance, whereas Karridale, Dinninup, Larisa, Daliak and Trikkala were the most resistant to the Western Australian *P. clandestina* isolate tested at that time when the race status of *P. clandestina* was unknown. Subsequently, You et al. (2005c) screened 101 isolates of *P. clandestina* from Western Australia on 9 subterranean clover varieties and were able to characterise a total of eleven races (in contrast to the 5 recognized previously). These races were defined and differentiated using octal nomenclature, that presented not only the first clear picture of the racial distribution of *P. clandestina* in Western Australian, but provided a sound basis for follow-up studies and future race designations. Races 173 and 177 in this study were widely distributed and were the most common races in Western Australia, and together constitute 80% of the isolates characterized. While 6 of the 7 subterranean clover host differentials were resistant to isolates belonging to race 001 and all were resistant to race 000, it is of concern that only 1 differential was resistant to race 157 and race 173 and that none of the host differentials were resistant to 177 (You et al. 2005d). Their approach facilitated, for the first time, rapid recognition and characterization of *P. clandestina* races, including their pathogenicity in relation to the differentials. However, these race studies were based on a survey of isolates collected more than 10 years ago. Hence, 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 rapidly generates new races that overcome existing variety resistances. Ma et al. (2009) analysed 61 isolates of *P. clandestina* belonging to eight pathogenic races using ITS region and the -tubulin gene of *Phytophthora* utilizing DNA sequencing and single strand conformation polymorphism (SSCP) technologies. Among genes/regions they analysed, the β-tubulin gene displayed a high degree of variability between isolates of *P. clandestina* and was further used to characterize the races in this species. Cluster analysis of pairwise distance matrix computed from SSCP profiles grouped the 61 isolates into two main clusters (A and B). Cluster A was further differentiated into 3 sub-clusters (I, II and III), within which isolates from race 177 formed sub-cluster I, isolates from race 000 formed sub-cluster II, and isolates from races 001, 044, 101, 143 and 157 together formed sub- cluster III. Cluster B contained all 31 *P. clandestina* isolates from race 173. The high degree of genetic variability and diversity we found within *P. clandestina* suggests that the pathogen evolves rapidly, and why there will likely be further proliferation of new races as a consequence of the current introduction of subterranean clover varieties with major gene-based resistance to this disease. In addition to characterization of intra-species variation in *P. clandestina*, SSCP of β-tubulin also successfully differentiated between races 173 and 177, the two most prevalent and most pathogenic of the races studied and which require a wide range of host differentials for conventional race identification. This was the first report, not only of the successful use of the β-tubulin gene to characterize races of *P. clandestina*, but also for any *Phytophthora* species. Their approach opened up the opportunity not only to study intra-species variation and races in other oomycete species adversely affecting pasture legumes, but to also monitor the evolution, distribution and spread of their races. Recently, Ma et al. (2010) defined the infection processes and the involvement of defense related genes in the expression of resistance in varieties of subterranean clover to *P. clandestina*. They demonstrated major differences in infection progression observed post-penetration between compatible and incompatible interactions. In susceptible cv. Woogenellup, hyphae grew into the vascular bundles and produced intercellular antheridia and oogonia in the cortex and stele by 4 days post inoculation (dpi), oospores in the cortex and stele by 8dpi, when sporangia were evident on the surface of the root. Infected taproots were discoloured. Roots of resistant cv. Junee showed no oospores or sporangia and no disease at 8 dpi. In gene expression studies Ma et al. (2010) using two races of *P. clandestina* to inoculate three varieties of *T. subterraneum* showed that three genes known to be associated with plant defense against plant pathogens were differentially expressed in the roots during compatible and incompatible interactions. Phenylalanine ammonia lyase and chalcone synthase genes were activated 4h post- inoculation (hpi) and cytochrome P450 trans-cinnamic acid 4-monooxygenase gene was activated 8hpi in the incompatible interactions in cvs Denmark and Junee following inoculation with Race 177. In contrast, in compatible interactions in cv. Woogenellup, there were no significant changes in the activation of these three genes following inoculation, indicating that these three genes were associated with the expression of resistance to Race 177 of the pathogen by the host.

<u>Western Australia - *Aphanomyces:*</u> In the high rainfall areas of south-west Western Australia, Ma et al. (2008) recently surveyed the incidence of an *Aphanomyces* sp. and *Phytophthora clandestina*. Of the 44 locations they assessed in that survey, an *Aphanomyces* sp. was detected at 23 locations with 14 of these locations also containing *P. clandestina*. Both *Aphanomyces* sp. and *P. clandestina* sporangia were found in the diseased roots of a single plant. This occurred in eight individual plants across six field sites. However, following on from this, of particular importance were the studies by O'Rourke et al. (2010) who conducted experiments to determine the species of *Aphanomyces* causing root disease on subterranean clover in the high rainfall areas of south-west Western Australia. The effects of flooding, temperature and inoculum concentration on the development of root disease on subterranean clover caused by this *Aphanomyces* sp. were also investigated as was its host range. Morphological and molecular characteristics were used to identify this pathogen as a new species *Aphanomyces trifolii* (O'Rourke et al. 2010). *A. trifolii* causes significant lateral root pruning as well as hypocotyl collapse and tap root disease of subterranean clover. The level of disease was greater in treatments where soil was flooded for 24h rather than for 6h or in unflooded treatments; and greater disease occurred at 18/13^{o}C than at the lower 10/5^{o}C or higher 25/20^{o}C. *A. trifolii* also causes root disease on annual medic (*M. polymorpha* and *M. truncatula* (O'Rourke et al. 2010). It is likely that the *A. euteiches* reported causing root disease on subterranean clover in Victoria was also *A. trifolii* and Victorian sites should be reassessed as *A. trifolii* now appears to be a major root pathogen across the higher rainfall areas of southern Australia, particularly where periodic flooding occurs as this predisposes subterranean clover to severe root disease from this pathogen. It is now clear that *A. trifolii* is a serious pathogen not only in the high rainfall areas of south-west Western Australia, but is a likely significant cause of severe root disease and subsequent decline in subterranean clover pastures across southern Australia.

South Australia: In comparison to Western Australia, Victoria and NSW, there have been very few reports of investigations undertaken in South Australia in relation to causes of root disease in subterranean clover. In South Australia Ludbrook et al. (1953) studied the "bare-patch disease" and associated problems in subterranean clover pastures and implicated *R. solani* as a cause of root rotting.

Relative importance of root pathogens: Root rot of clovers clearly involves a complex of fungi which interact not only among themselves but also with the biotic and abiotic environment surrounding them. A range of different fungi are normally associated with diseased subterranean clover roots and no one fungus has been able to reproduce the wide range of different field disease symptoms observed in different locations. Investigations which implicate a single fungus as the cause of the disorder have often been conducted solely with that particular fungus. That a number of different fungi are present on diseased roots, and that they do interact to cause enhanced disease has been clearly demonstrated and more research needs to be conducted into these interactions and associations. We believe that at the current state of investigations the most important fungi associated with subterranean clover root rot are, in order of importance, *Phytophthora clandestina*, *Pythium irregulare*, *Aphanomyces trifolii* and *Rhizoctonia* spp. In comparison with these 4 pathogens, it now appears that *F. avenaceum* is in fact of much lesser importance as a cause of root disease on annual clovers than first thought some decades ago.

9.2.1.5 Soil-borne nematodes

Across southern Australia – causal species and impact: *Meloidogyne* sp. has been recorded to cause extensive damage to subterranean clover in South Australia (Ludbrook et al. 1953) and *M. hapla* and *M. javanica* have both been implicated as a cause of poor growth of subterranean clover in NSW (Colman 1964). Powell (1971) demonstrated that significant interactions occur between nematodes and fungi in root disease complexes. A wide range of nematode genera have been recorded in association with subterranean clover roots, including *Aglenchus, Aphelenchus, Ditylenchus, Filenchus, Meloidogyne, Merlinius, Neopsilenchus, Nothotylenchus, Pratylenchus, Rotylenchus* and *Tylenchorhyunchus* (Khair 1981), but only a few of these are likely to be significant pathogens. *Meloidogyne javanica*, *M. hapla*, *M. incognita, Pratylenchus* sp., and *Radopholus* sp. were considered potential subterranean clover pathogens in Western Australia (Shipton 1967b). Subsequent surveys of subterranean clover root rot sites in Western Australia indicated the presence of *Meloidogyne*, *Heterodera, Pratylenchus, Trichodorus* and *Radopholus* (Pung et al. 1988) in situations with strong evidence for a significant role played by nematodes in root disorders. For example, Pung et al. (1991a) investigated the role of *Meloidogyne arenaria* and root rot fungi in the decline of subterranean clover in infested fields by soil application of the fungicide, benomyl, and the nematicide, aldicarb. *M. arenaria* appeared to be a significant cause of poor productivity of subterranean clover, as aldicarb inhibited root-knot nematodes and increased plant vigour. The study by Stirling and Lodge (2005) of nematode populations in subterranean clover pastures in two regions, the New England, NSW and south-east of SA provides a contrast. There, plant-feeding nematode population densities found at the time of sampling were not considered economically significant. Endoparasites, *Pratylenchus*, *Heterodera* and *Meloidogyne,* were found in the north but only *Pratylenchus* in the south. The population densities of some ectoparasites were greater, though not considered problematic. The most compressive work on root-knot nematodes and their association with fungal root rots in subterranean clover was undertaken in Western Australia by Pung and colleagues. Pung et al. (1988) investigated *Meloidogyne* spp. in subterranean clover in the lower south-

west of Western Australia and found *Meloidogyne arenaria* the 12 study sites. This was the first record of *M. arenaria* on subterranean clover in Australia or elsewhere. They demonstrated a clear negative relationship between gall and root rot indices of the tap roots. Although pathogenicity tests with *M. arenaria* at five inoculum levels (0, 800, 1600, 4000 and 8000 eggs per 250 ml pot) did not demonstrate clear pathogenicity, they showed that it is pathogenic at higher inoculum levels (> 16000 eggs per pot). Pung et al. (1992b) conducted pot experiments to examine the effects, on the root health and growth of subterranean clover, of *F. oxysporum* and *M. arenaria* applied singly or in combination. They showed that while *M. arenaria* had no effect on root necrosis in both the pasteurised soil and autoclaved soil, it still could adversely affect plant growth. Further, Pung et al. (1991b) demonstrated that the timing of infection and the proximity of root tips of the host root system to infection by *M. arenaria* and *F. oxysporum* appeared to be the major determining factors of root growth and of disease development in plants exposed to these pathogens. For example, they showed that the induction of galls by the nematode and early infection by *F. oxysporum* resulted in a severe inhibition of root growth, particularly of the lateral roots. In sequential inoculation with *F. oxysporum* or *M. arenaria*, the organism added 2 weeks later had little or no effect on root development. The first organism (*M. arenaria* or *F. oxysporum*) to infect the germinated seedlings was the main cause of root growth inhibition. Concurrent infection by *F. oxysporum* and *M. arenaria* resulted in less *M. arenaria* gall production on the tap root system than those exposed to the nematode alone or in advance of the fungus. The effect of environmental factors on *M. arenaria* and its infectivity on subterranean clover was also investigated both in a naturally infested subterranean clover pasture and under controlled conditions in a pot experiment (Pung et al. 1992a). In the field, the nematode population density was affected by seasonal changes. The hatching of *M. arenaria* was determined by the germination of subterranean clover brought about by the opening seasonal rains in April or May. The first generation of *M. arenaria* in subterranean clover roots appeared to develop and reproduce more rapidly while the soil temperature was still relatively high (> 15°C) in May- June and the second generation developed as soil temperature increased between September and November. These findings were consistent with observations from a pot experiment, where *M. arenaria* gall production and its development and reproduction in both the tap and lateral roots were greater at 20/15°C and 25/20°C than at 15/10°C. These studies showed that the timing of the opening seasonal rains and soil temperatures are important determinants of the severity of disease caused by *M. arenaria*. Greater nematode infection in the tap roots occurred at moisture levels of pF 1.28 and 0.97, but not at
0.71, and this may be related to better nematode mobility at the higher soil moisture contents and its preference for tap roots under more favourable conditions. While Kouame et al. (1989) screened subterranean clover germplasm for resistance to *Meloidogyne* species, there is scope to now both identify new resistances to nematode disorders and to screen current varieties for their reaction to such disorders.

9.2.1.6 . Current Management Strategies - Disease management using chemicals

__Victoria:__ Various attempts have been made to try to find ways of controlling root rots in subterranean clover in Victoria. Taylor et al. (1985a) controlled root disease and increased subterranean clover yield from a pre-germination soil treatment with metalaxyl + benomyl. In pot tests they showed that either metalaxyl or phosethyl Al treatments would control root rot. Kellock (1975) demonstrated that benomyl was fungicidal to *F. avenaceum* in laboratory tests. Greenhalgh (1983) showed that metalaxyl controlled root disease caused by *P.*

irregulare. Greenhalgh and Clarke (1985) used metalaxyl, benomyl, or metalaxyl + benomyl drenches to reduce both root rot severity and the incidence of *Pythium* spp. and *F. avenaceum* on subterranean clover roots. Smiley et al. (1986) showed that root rots in subterranean clover could be reduced by treatment of seeds with fungicides, metalaxyl or benomyl or by drenching soils with these same fungicides. Subsequently, Hochman et al. (1990) and Burnett et al. (1994) confirmed that metalaxyl could provide useful control of root disease, especially when caused by *P. clandestina*, as did Greenhalgh et al. (1994) for applications of potassium phosphonate, primarily against this same pathogen.

Western Australia: While some of the early attempts to control root rot of subterranean clover using fungicides in Western Australia were not encouraging (*e.g.*, Barbetti 1983a,c, 1984b, 1985a) others were more promising (Barbetti et al. 1987b). Some increases in seedling survival and small reductions in root rot levels have been obtained before 1984 (e.g., Barbetti 1984a) and where seed treatments with fungicides were tested during two growing seasons in 1984 and 1985 in root rot-affected fields in Western Australia resulted in both large increases in seedling survival and decreases in the severity of decay of tap and lateral root systems. These treatments containing metalaxyl were the most promising; thiram and propamocarb were less effective, and benomyl and iprodione were ineffective (Barbetti et al. 1987b). In these same studies, decreases in the severity of rotting of tap and lateral root systems were also obtained from rhizobial inoculations. *Rhizobia* can ameliorate disease in legumes by improving nitrogen nutrition and by competing with the pathogen in the rhizosphere. In other investigations (Barbetti 1984a), metalaxyl and thiram showed useful potential for further development as possible commercial treatments for increasing seedling survival in susceptible varieties being resown into areas affected by root rot. Of the two chemicals, metalaxyl was the most promising. However, this result contrasts with the results (Barbetti 1985a) where six rates of metalaxyl seed treatments, had no significant effect on seedling survival, tap root rot, or root dry weight at two field sites in Western Australia. In a field trial conducted in a subterranean clover cv. Yarloop pasture at Harvey, Western Australia, a single spray of Foli-R-Fos 20% applied 8 days after the opening seasonal rains reduced both tap and lateral root rot severity and the incidence of *P. clandestina* on tap roots (MJ Barbetti, unpubl.). In contrast, various metalaxly seed and soil drench treatments had no significant beneficial effect on the number of plantsgerminated, root rot indices, or total plant dry weights (Barbetti 1983a,c, 1984b, 1985b). When fungicide drenches of benomyl, metalaxyl, iprodione, propamocarb or thiram applied to intact soil cores taken from known root rot-affected fields in Western Australia, metalaxyl was the most effective in reducing seedling damping-off (Barbetti et al. 1987a). It was noteworthy that in these studies that the most effective fungicide for reducing the level of rotting of both tap and lateral root systems of surviving plants varied from season to season at any one field site and varied between different field sites in any one season with each fungicide giving a significant reduction in root disease on at least one occasion. Such results strongly suggest that different individuals or complexes of root pathogens were operative between seasons in any one site, and between sites for any one season. In some instances it appears that different individual root pathogens or pathogen complexes were operative on tap roots compared to lateral roots. Such findings clearly demonstrate the difficulty in successfully managing damping-off and root disease in clover pastures in southern Australia with soil applied fungicides. In another study in Western Australia (Barbetti 1983a,c,), metalaxyl, benomyl and a metalaxyl/benomyl mixture, applied as soil drenches on an established subterranean clover pasture two weeks after the opening rains had no significant effects upon the levels of tap and lateral root rot, nor upon the total dry weight per plant. Even if a successful fungicide was found, drenches would not be practical or economic. While fungicide treatments are not effective enough on their own to make their use economically justifiable for very susceptible varieties, it is possible that they may have a place in an integrated control system incorporating varieties with at least some resistance to root rot. Further, as there is a range of soil-borne and foliar pathogens that can be found on see of subterranean clover (Barbetti 1990c), application of fungicide may offer a wider advantage when re-sowing pastures than for just the particular soil-borne pathogen targeted. It is clear that there exist significant but rarely exploited opportunities for utilizing low cost chemical seed treatments to ensure successful stand establishment. While Apron® is registered for control of damping-off in some pasture legumes, this chemical is expensive and targets only one of several pathogen components associated with damping-off. There are many alternative fungicides treatments that not only would be more effective as they target a much wider range of soil-borne pathogens associated with damping-off, but also would be much cheaper, encouraging wide usage. The current poor level of utilisation of cheap and available chemicals for both soil-borne and foliar disease management by farmers is due to lack of knowledge of their likely benefits. There is also the challenge that cheaper and more effective possibilities simply have not been identified and/or evaluated for their potential roles in fungal, oomycete, or viral disease management.

9.2.1.7 Current Management Strategies - Disease management using cultural practices

Victoria: In Victoria, Smiley et al. (1986) showed that simulated cultivation of soil in cores could also significantly reduce root rots in the dryland pasture soil that had little surface litter, but not in the irrigated pasture soil which had high levels of organic debris (and pathogen inocula) that was distributed through the surface layer of the soil profile. Smiley et al. (1986) also demonstrated that root rots could be reduced by treatment of seeds with *Rhizobia* on legumes.

Western Australia: Field experiments in the 1970s in south west Western Australia demonstrated that various cultivation and cultural practices can significantly reduce the levels of tap and lateral root rot for up to two seasons following treatment application (Barbetti and MacNish 1984). The best treatments were those of fallowing an area from August to March before cultivation and reseeding, or spring cultivation before sowing to oats followed by a March cultivation and reseeding. However, due to lack of long-term persistence of root rot reductions, high levels of root rot still remaining even after treatment, concern over reduced stand densities, production losses from fallowing and increased damage from root knot nematodes following cultivation, no practical cultivation or cultural practice treatment was recommended to farmers as a means of reducing root rot severity. Small reductions in root rot severity have been obtained from inoculating seed with *Rhizobia* (MJ Barbetti, unpubl.) and a strain of *Rhizobium trifolii* significantly reduced root rot caused by *F. avenaceum* in glasshouse studies (Wong 1986). In two field trials in Western Australia (Barbetti 1991a), complete removal of subterranean clover for one season or, in particular two seasons, significantly reduced tap and lateral root disease in the immediate following year in which subterranean clover was allowed to regenerate. However, the second season of regeneration these effects were either small or absent. Subterranean clover removal had greater effect on reducing lateral root disease than tap root disease in regeneration pastures. It is noteworthy that there were often large increases in plant size in regenerating pastures following complete removal of subterranean clover for one season or, in particular, two consecutive seasons. This effect also persisted poorly beyond the first season of regeneration. Unfortunately, the losses in terms of subterranean clover herbage and seed yield during the period of subterranean clover removal were not offset by subsequent benefits from root disease reductions, as there was no corresponding increase in total herbage production. Removal of subterranean clover for short periods (1 or 2 years) as an agronomic practice may be useful in overcoming root rot problems associated with this species in the high (>750 mm) rainfall zone, the zone where severe root rot most frequently occurs in Western Australia, providing a suitable alternative pasture species can be cultivated and grown during the non-clover phase. Barbetti (1990a) showed that the levels of root disease could also be modified through application of lime to increase soil pH. This prospect warrants further investigation. While manipulation of cultural practices is not effective enough on its own to make their use economically justifiable for very susceptible varieties, it is possible that they may have a place in an integrated control system incorporating varieties with at least some resistance to root rot.

9.2.1.8 Current Management Strategies - Disease management using host resistance

The most promising and cost-effective avenue for disease control clearly has and remains the development and use of varieties with increased field resistance to root rot.

<u>*Western Australia – subterranean clover – resistant varieties:*</u> Extensive field testing of subterranean clover varieties for root rot resistance in root rot-affected areas of Western Australia had been conducted (Gillespie 1979, 1980, 1983a). Even by the early 1980’s, more than 500 introductions and crossbreds have been tested and a wide range of resistance has been observed. However, no fully resistant clovers were identified at that time although many tolerant lines were found then (Gillespie 1983b) and subsequently. Varieties with good field resistance to root rot are Daliak (Gillespie 1983a; Nichols et al. 1996), Dinninup (Gillespie 1983a; Nichols et al. 1996), Esperance (Nicholas 1980b; Nichols et al. 1996), Junee (Nicholas 1985a), and Karridale (Nicholas, 1985b). Larisa was known to have a moderate degree of field root rot resistance (Nicholas 1980a) while Trikkala had less but still useful resistance (Nicholas 1980c). Varieties Denmark (Nichols and Barbetti 2005b) York (Nichols and Barbetti 2005h) and Goulburn (Nichols and Barbetti 2005d), all released in the 1990’s, all had resistance to the most commonly occurring race of *P. clandestina*. Variety Gosse (Nichols and Barbetti 2005c) released in the 1990’s, Riverina (Nichols and Barbetti 2005f), along with the most recently released varieties of Napier (Nichols et al. 2006a; Nichols and Barbetti 2005e) and Coolamon (Nichols et al. 2007a; Nichols and Barbetti, 2005a), all have good resistance to more than one of the most common races of *P. clandestina*. Unfortunately, the recently released variety, Urana, is quite susceptible to the two most commonly occurring races of *P. clandestina* (Nichols et al. 2006b; Nichols and Barbetti 2005g). In attempts to refine methods of screening subterranean clover varieties for resistance to root rot Barbetti et al. (1986b) screened 12 commonly grown varieties both under controlled conditions for their resistance to five pathogens (*F. avenaceum*, *F. oxysporum*, *Phoma medicaginis*, *Pythium irregulare* and *R. solani*) commonly associated with root rot, and under field conditions for their resistance to natural root infections. All varieties showed decreased seedling survival (particularly from *P. irregulare* and *R. solani*), tap and lateral root rot (particularly from *F. avenaceum*, *P. irregulare* and *R. solani*) and reduced plant size (particularly from *R. solani* and *P. irregulare*). Individual varieties generally differed in their response to the five pathogens and for any one pathogen there was generally a range of variety susceptibilities. Varieties with the best resistance to individual root pathogens were identified. However, the results for the five individual pathogens under controlled environment conditions only showed correlation for field data for some of the parameters measured. You et al. (2005c) also screened subterranean clover breeding lines for resistance to root rot caused either by *F. avenaceum* or *P. irregulare*. Only one of the

tested lines showed good resistance to root rot caused by *P. irregulare* but this was a significant finding as effective resistance to this pathogen is extremely rare. High levels of resistance to *F. avenaceum* were identified in 8 midseason lines and 5 late season lines of *T. subterraneum* ssp. *subterraneum* and 6 late season lines of *T. subterraneum* ssp. *yanninicum*. These sources of resistance will be of significant value to breeding programs aimed at developing new more resistant varieties to this particular pathogen as few sources of useful resistance in subterranean clover to *P. irregulare* have been identified. Significantly, seedling survival in 14 late season and 15 midseason lines of *T. subterraneum* ssp. *subterraneum* and in 1 line of late season *T*. *subterraneum* ssp. *yanninicum* were not adversely affected by *F. avenaceum*. Similarly, seedling survival in 5 late season and 6 midseason lines of *T. subterraneum* ssp. *subterraneum* was not adversely affected by *P. irregulare*. More importantly, they found 4 late maturing and 6 mid-season *T. subterraneum* ssp. *subterraneum* lines that showed no significant reduction in seedling survival in the presence of either *P. irregulare* or *F. avenaceum* and genotypes carrying such seedling survival resistance to multiple pathogens is rare. It is noteworthy that specific resistance to root disease caused by either of the individual pathogens was not linked to survival levels of the seedlings. This indicates that selection of lines for field performance should not only rely on specific resistance to root disease but also on overall ability or tolerance of seedlings to survive in the presence of these pathogens, making management of disease complexes even more challenging. Resistance identified to either pathogen could be utilized in developing new varieties for areas prone to the root rot caused by these pathogens or utilized as parental materials in breeding programs. While some useful resistance to *P. irregulare* has now been located, a key priority is to locate highly effective sources of resistance to this pathogen as without such resistance annual clovers have little chance of actually successfully emerging in regions conducive to root disease caused by this pathogen.

<u>*Western Australia – subterranean clover – dealing with the challenge Phytophthora clandestina races:*</u> You et al. (2005b) screened 84 genotypes, including 71 ssp. *subterraneum* and ssp. *yanninicum* breeding lines of subterranean clover and 13 commonly used varieties for resistance to root rot caused by two races of *P. clandestina* that occur most widely in Australia. Resistance to race 001 was identified in seven mid-season genotypes of ssp. *subterraneum*, including the new variety, Coolamon and one genotype also showed resistance to race 373. Of the late flowering ssp. *subterraneum* genotypes tested,

13 showed resistance to race 001 and four of them also showed resistance to race 373. In the late flowering ssp. *yanninicum* group 12 of 13 genotypes tested, including the new variety, Napier (Nichols et al. 2006a), showed resistance to both races. Of the midseason ssp. *yanninicum* genotypes all but two of 19 tested showed resistance to both races. The resistance observed in the majority of ssp. *Yanninicum* and in some ssp. *subterraneum* genotypes, indicates that these are useful sources of resistance that can be exploited, either directly as new varieties to minimise damage from this disease or as parents in

breeding programs to develop varieties with improved resistance to *P*. *clandestina*. This study established the availability of 51 advanced lines and 11 varieties as sources of resistance against *P. clandestina* race 001 and 36 lines and 4 varieties for race 373, among these 36 lines and 4 varieties were resistance against both races. Subsequently, You et al. (2006) demonstrated that cv. Denmark was highly resistant to races 001, 101, 141, 151 and 143, and moderately resistant to race 121, while cv. Meteora was highly resistant to race 151, moderately to highly resistant to races 001 and 101 and moderately resistant to races 121, 141 and 143. You et al. (2005d) characterised a total of eleven races (in contrast to the 5 recognized previously) presented the first clear picture of the racial distribution of *P. clandestina* in Western Australia and this will be important to farmers in relation to the deployment of appropriate resistances in different regions. It is now clear that clearly identified race distribution differences between regions across southern Australia is critical to and provides the only sound basis for the selection/breeding and deployment of appropriate varieties for specific regions of Australia to counter the predominant race populations in each region. Studies, such as that by You et al. (e.g., You et al. 2006), constitute one of the few research investigations ever undertaken demonstrating a clear evolution in pathogenic specialization of a pasture pathogen in response to the use of specific varieties or spectrum of varieties of a specific pastures species in a given geographic region. It is vital that if we are to manage the significant threat posed to subterranean clover by *P. clandestina*, then the race situation for this pathogen which has the ability to rapidly generate new races to overcome existing variety resistances needs to be urgently reassessed across southern Australia.

<u>*Subterranean clover – southern Australia needs resistance to multiple pathogens:*</u> While identifying sources of resistance to individual root pathogens allows the development of varieties with further enhanced root rot resistance, ideally, identification of resistance to more than one root and/or foliar pathogen in a single line or variety should now be a priority. That this can be achieved was demonstrated by You et al. (2005a) when screening 100 subterranean clover genotypes including 72 advanced breeding lines from *T. subterraneum* ssp. *subterraneum* and *T. subterraneum* ssp. *yanninicum* and 28 *T. subterraneum* commercial varieties were screened in the field for resistance to race 2 of *Kabatiella caulivora*, and the resistances found were related to known resistance to major root pathogens in the region. The unique importance of that study was that, for 12 genotypes of subterranean clover, these resistances were related to those shown to major root pathogens, *viz*. one or more of *P. clandestina*, *P. irregulare*, and *F. avenaceum*. Availability of genotypes with such resistances to multiple pathogens is expected to be particularly valuable for the breeding/selection of subterranean clover in relation to the development of new varieties with effective resistance to a range of pathogens that commonly occur in southern Australian annual legume pastures. 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 not only with subterranean clover and also for all other annual and perennial pasture legume species.

<u>*Western Australia – other annual clovers*</u>*:* You et al. (2008) assessed 15 pasture legume genotypes for their resistance/susceptibility to five different zymogram groups (ZG) of the root rot pathogen *R. solani* under glasshouse conditions. *Ornithopus sativus* cv. Cadiz showed resistance to *R. solani* ZG 4 and ZG 11, while *T. michelianum* cvs. Frontier and Paradana; *O. sativus* cv. Marguita and *T. purpureum* cv. Electro showed resistance to ZG 11. All the tested genotypes suffered severe root rot (score >2) caused by *R. solani* ZG 1-5 and ZG1-4. *R. solani* ZG 6 caused the greatest death of legume seedlings followed by ZG 1-5 and ZG 11 and ZG 1-4. *R. solani* ZG 4 caused significantly less seedling mortality compared with all the other ZG s tested. Li et al. (2009a) screened 36 genotypes, including 15 varieties and 10 breeding lines of *T. subterraneum*, and including a single genotype of each of 7 other species of *Trifolium* (viz. *T. dasyurum, T. glanduliferum, T. incarnatum, T. michelanium, T. purpureum, T. spumosum* and *T. vesiculosum*), under controlled environment conditions for resistance to root disease caused by the most pathogenic race of *P. clandestina* occurring in Australia, *viz*. race 177. This was the first time these other *Trifolium* species other than *T. subterraneum* had been screened for their response to *P. clandestina*. The root disease caused by *P. clandestina* was the first report of susceptibility to this pathogen for the seven other species of *Trifolium*. While within *T. subterraneum*, a very high level of resistance was identified as expected in cvs Denmark, Junee and Meteora (scores ≤1.3 [0-5

scale where 0 = no disease] across 2 separate screening tests) and in the breeding lines SL027 and SM023 (scores ≤1.1 across 2 separate screening tests), six of the seven of the 7 other *Trifolium* spp. (viz. *T. dasyurum, T. glanduliferum, T. incarnatum, T. michelanium, T. purpureum, and T. spumosum*) showed a high level of resistance (scores ≤0.7 across 2 separate screening tests), while *T. vesiculosum* showed a disease score of ≤1.7 across both screening tests. The high levels of resistance identified against *P. clandestina* are useful sources of resistance that can be exploited commercially, either directly to minimise damage from this disease or as parents in breeding programs to develop varieties within the genera/species tested with improved resistance to this highly pathogenic race of *P. clandestina*.

9.2.1.9 What are the gaps and research needed?

The critical gaps that need addressing in relation to soil-borne diseases of annual clovers for southernAustralia are as follows:

Identify highly effective sources of resistance across all clovers & medics to *Pythium irregulare*

Define races of *Phytophthora clandestina* across southern Australia

Define resistances of current & impending varieties to *P. clandestina* races

Identify general tolerance in clovers to a wide cross section of *P. clandestina* races

Identify resistances of current & new varieties to *Aphanomyces trifolii*

Develop effective seed treatments to ensure successful reseeding and re-establishment

Determine incidence and impacts of fungal diseases in non-sub clover pastures and nematode disorders in across all pastures

9.3 Foliar diseases of annual clovers

9.3.1 Introduction

Fungal foliar diseases of clovers have not been comprehensively reviewed since the earlier Australian reviews by Barbetti and Sivasithamparam (1986) and Johnstone and Barbetti (1987), or reviews have only focused on a single pathogen such as *Kabatiella caulivora* (e.g., Barbetti 1996).

Johnstone and McLean (1987) reviewed viral diseases of subterranean clover in detail, and they were also included briefly in subsequent general reviews, the last of which was in 2004 (Johnstone and Barbetti 1987; Panetta et al. 1993; Jones 1996, 2000, 2004a).

9.3.2 Fungal foliar diseases

As a relatively small number of fungal foliar diseases are or overriding importance on annual clovers in Australia, other fungal pathogens that can sometimes be important on annual clovers will not be discussed in detail in this review other than for the comments immediately below on these relatively minor fungal diseases, *viz*.:

Pseudopeziza leaf spot or common leaf spot: Pseudopeziza leaf spot or common leaf spot is a common but normally minor disease of clovers caused by *Pseudopeziza trifolii* (Valder 1954; Anon. 1975a; Barbetti 1983d). The fungus causes brown to black spots, usually on the leaf blade but occasionally on the petioles and stems. While the disease can be severe under conditions of infrequent grazing it is normally of little economic significance (Valder 1954; Barbetti 1983b).

Clover blackstem and leaf spot: Clover blackstem and leaf spot, caused by *Phoma medicaginis* is a very common but rarely damaging disease of subterranean clovers, though occasional isolates are quite pathogenic (Barbetti 1983d). However, most isolates are pathogenic to and can be more damaging on some other clovers such as strawberry clover (*T. fragiferum*) (Barbetti 1983d). This disease causes brown to black leaf spots and petiole lesions and has also been blamed for a marginal leaf scorching condition (Barbetti 1985d). The fungus is seed-borne and can survive in soil debris for at least two years.

Pepper spot: Pepper spot is a very common disease of clovers caused by the fungus *Leptosphaerulina trifolii* (Walker 1956a; Shipton 1967a; Anon. 1975a; Barbetti 1983d). The disease is so named because of the many small brown to black spots which occur on the leaves and petioles. The disease is usually more severe on the older leaves and can cause a leaf scorch symptom (Anon. 1964a). Disease development occurs during cool moist weather (Shipton 1967a). Usually pepper spot is of little economic importance (Walker 1956a; Barbetti 1983d).

Wart disease: Wart disease, caused by the fungus *Physoderma trifolii*, occurs particularly on clovers growing in wet situations (White et al. 1956; Shipton 1967a; Anon. 1975a; Barbetti 1983d). Affected plants show swellings on the stems, petioles, leaves and flowers. In severely affected plants the galls often unite to cause distortion and twisting of affected parts. This disease is generally considered to be of little economic significance (Shipton 1967a). Outbreaks can be avoided by improving the drainage in low-lying areas, thus making conditions unsuitable for the fungus.

Sclerotinia: Sclerotinia, caused by *Sclerotinia trifoliorum*, is seen as a soft rot of the crown and roots of clovers and less frequently, the basal part of the stems. The new growth of infected plants wilts and the dead tissues can be overgrown by the white fungal mycelium in very moist conditions (Shipton 1967a; Barbetti 1983d). Pastures affected are usually thick and lush (Walker 1956a). Some control may be achieved by grazing affected pastures, presenting lush growth and so avoiding favourable disease conditions of high humidity (Walker 1956a).

Stemphylium leaf spot: Stemphylium leaf spot, caused by *Stemphylium botryosum*, *Stemphylium sarciniforme*, and possibly other *Stemphylium* spp., can also be found on clovers. The symptoms are irregular, pale or dark brown sunken spots which may develop into irregular concentric light and dark brown lesions which usually appear first on the older leaves. This disease is rarely of any economic significance (Valder 1954; Anon. 1975a; Barbetti 1983d).

Stagonospora leaf spot: Stagonospora leaf spot, caused by *Stagonospora* spp. also occurs on clovers. Symptoms include large spots (up to 10mm in diameter) white to pale brown with a darker brown margin. Pycnidia readily develop in the lesions. This disease does not cause serious loss of herbage (Walker 1956a; Anon 1964; Anon. 1975a).

Downy mildew: Downy mildew, caused by the fungus *Peronospora trifoliorum*, occurs on clovers particularly in the cool moist conditions of spring. Symptoms include a purplish-grey fungal mould on the under surface of leaves. As the disease develops the leaves turn yellow. This disease rarely causes serious damage.

Anthracnose: Anthracnose, caused by the fungus *Colletotrichum trifolii*, can occur on most clovers but red clover is particularly susceptible to attack (Butler 1953). The first and most conspicuous symptoms of this disease are drooping leaflets and flower heads and dark lesions on the stems (Valder 1954). Affected plants become defoliated, the clover petioles turn brown, and, in cases of severe infection, a crown rot condition develops (Butler 1953; Anon 1975a). The disease is spread by seed contamination and is carried over from season to season in diseased remains and on or in soil (Butler 1953). Spores are spread within a stand by rain splash, wind, and also possibly by insects and animals (Butler 1953). The disease is favoured by warm humid weather (Anon. 1964a) and historically there have been destructive outbreaks in coastal areas of NSW (Valder 1954). On highly susceptible species the stand should be cut and the hay removed as soon as infection becomes apparent (Butler 1953).

Ascochyta: Leaf spot, black stem and stem decay caused by *Ascochyta* spp. has been recorded on red and subterranean clovers in NSW and at least one severe outbreak has been reported (Valder 1954). *Ascochyta pisi* has been recorded as a cause of leaf spotting and stem decay on subterranean clover (Shipton 1967a). *Mycosphaerella carnithiaca* has been recorded as a cause of a leaf spotting condition in red clover (Anon. 1964a; Anon. 1975a).

9.3.3 . Disease Symptoms – Important fungal diseases

<u>*Clover scorch:*</u> Clover scorch (*Kabatiella caulivora*) is most frequently seen in dense, ungrazed, clover- dominated swards. The fungus can invade any part of the foliage, but most commonly infects the area where the leaflets join the petioles (Bokor 1972). This causes the earliest symptom of the disease, the characteristic turning of the leaves to expose the under surface. Brown lesions occur anywhere on the petioles, stems, runners and peduncles. Infection destroys the vascular tissue and the leaves wilt and desiccate. As the disease progresses, the clover stand ‘opens up’. In severe cases, entire affected paddocks appear ‘scorched’. The sudden collapse of an infected stand may occur during periods of high evaporation demand in spring.

<u>*Cercospora:*</u> Cercospora (*Cercospora zebrina*) causes salmon pink or brown leaf spotting on subterranean clover and drooping flowered clover (Barbetti 1983d,e,f). Affected areas can coalesce to cause leaf collapse. The fungus also attacks the stalks of the leaves and flowers, sometimes girdling them and causing collapse.

Rust: Rust diseases are characterised by the presence of slightly raised reddish brown particles on the leaflets and petioles (Valder 1954). Affected leaves frequently wither and die causing considerable injury and sometimes the death of the plant (Valder 1954; Anon. 1964a).

Powdery Mildew: Powdery mildew, caused by the fungus *Erysiphe polgonii*, typical causes white mildew growth on foliage and with leaf scorching and collapse where symptoms are severe.

9.3.4 Scope, extent and impact of important fungal foliar diseases on pasture productivity

Clover scorch – Victoria: Clover scorch caused widespread damage across Victoria during the late 1960's and early 1970's (Kellock 1971). For example, in 1974 an estimated 40,000 ha of subterranean clover in Victoria were severely affected, and similar areas have been infected in other years, with severe infections usually causing nearly total loss of the pasture either for hay, seed or grazing (Clarke, 1980).

Clover scorch – NSW: Clover scorch was first recorded in Australia in 1955 on subterranean clover in the Berrigan irrigation area of NSW (Walker 1956a,b) and where severely affected showed up to 90% reduction in yield (Walker 1956b). Clover scorch caused particularly widespread damage across NSW during the late 1960's and early 1970's (Stovold 1976a).

Clover scorch – South Australia: Clover scorch caused widespread damage across South Australia during the late 1960's and early 1970's (Beale 1972, 1976). In South Australia, clover scorch in the early to mid-1970's affected some 800,000 hectares, 300,000 hectares seriously, and on some farms livestock carrying capacity was reduced by over 25% (Beale 1972, 1976).

Clover scorch – Western Australia: Clover scorch was widespread across Western Australia during the late 1960's and early 1970's (Bokor 1972). Clover scorch was first recorded in Western Australia in 1962, but it was not until the early 1970's that numerous severe outbreaks were reported, with complete collapse of subterranean clover stands on some farms (Bokor 1972). In seasons which favoured the disease, hay production was reduced by as much as 50% and grazing capacity by as much as 30% at sites with susceptible varieties. Three extreme examples of losses in Western Australian from clover scorch include, (i), a 92% decrease in the variety Woogenellup seed yield, (ii), a 90% herbage yield reduction and almost no seed set in Yarloop, and (iii), a 66% reduction in plant density of the variety Yarloop in the season following a clover scorch outbreak (Gillespie 1983a). Even the partially resistant variety, Larisa, can suffer up to 62% loss of seed yield (Barbetti 1987a). Clover scorch has most commonly affected the varieties Seaton Park, Woogenellup and Yarloop. Seed production losses are important in the self-regenerating annual pastures in which subterranean clover is used. High levels of disease reduce seed reserves and lead to the failure of the subterranean clover component, with the pasture becoming dominated with less desirable species. The greater the effect on seed production the greater the reduction in productivity and persistence of susceptible subterranean clover varieties, leading eventually to severely deteriorated (i.e. weedy, low nitrogen fertility) pastures that have to be resown (Chatel et al. 1972; Bokor and Chatel 1973; Bokor et al. 1978a,b). In 1990, a new race of clover scorch appeared in the field at Denmark, on the south coast of Western Australia (Barbetti 1995c). This race was capable of attacking many previously highly resistant varieties such as Esperance, Green Range and Junee, and other field resistant varieties such as Mt Barker and Karridale, widely sown throughout southern Australia. Approximately 4 million hectares of subterranean clover pasture in southern Australia remain at great risk if the new clover scorch race was to become more widespread across southern Australia. Strategies were implemented in order to slow its spread and impact, and while has remained restricted to a geographical region surrounding Esperance on the south coast of Western Australia (Barbetti 1995c). However, while the original race, designated race 1 (Bayliss et al. 2001a,b), continues to predominate across the high rainfall subterranean clover growing areas of southern Australia (MJ Barbetti, unpubl.), race 2 is also expected to spread across wider areas of southern Australia in coming years (MJ Barbetti, unpubl.).

Cercospora – eastern Australia: In eastern Australia *C. zebrina* has been a known pathogen of clovers for many years (Valder 1954; Anon. 1964a, 1965) but there it is generally considered to be of only minor economic significance (Valder 1954; Anon. 1975a).

Cercospora – Western Australia: In Western Australia, since the disease was first discovered there in
1980 (Bokor 1982, 1983; Barbetti 1983d,e,f, 1985b, 1991b) it has become both more widespread (Barbetti 1983d,e,f) and has caused severe defoliation and reduced seed production in very susceptible varieties such as Esperance, Daliak and Nungarin (Barbetti 1985b). Complete stand collapse has been observed in the very susceptible variety Esperance (Barbetti 1985b) and less severe infections have resulted in seed losses up to 58% (Barbetti 1983e,f). The highly susceptible subterranean clover varieties it causes severe losses in herbage and especially seed yield (Barbetti 1987a,b), demonstrates the need to ensure that future varieties for disease conducive environments have adequate resistance. Further, there are interactions between this pathogen and other diseases such as with clover scorch disease (Barbetti 1985c) and it is likely that the full impact of this disease has not been appreciated outside of Western Australia.

Rust – eastern Australia: Rust (*Uromyces trifolii-repentis*) occurs at any time of the year in any district but tend to be most severe when high humidity and warm temperatures prevail (Anon. 1975). While little data is available on the importance of rusts they are known to cause substantial losses in Victoria and NSW if infections are severe (Peterson 1954; Anon. 1975a). For, example, many decades ago, Valder (1954) reported rust as the most serious disease of subterranean clover in NSW, particularly in the tableland and coastland districts. Peterson (1954) observed serious losses from rust especially on cv. Mt Barker in NSW coastal districts and reported the death of entire clover stands at seedling or flowering stages, reduced clover growth by 60-70% and reduced seed yield and size. Fortunately severe outbreaks in most regions have been sporadic.

Rust – Western Australia: In Western Australia, rust is found on all clovers and are all caused by one or more species of *Uromyces* and particularly subspecies of the fungus *Uromyces trifolii* (Shipton 1967a) such as (*Uromyces trifolii-repentis*) (MJ Barbetti, unpubl.). While it can sometimes be devastating in conducive regions and seasons (Barbetti 1983d; MJ Barbetti, unpubl.), very severe outbreaks in most regions have been more sporadic (Barbetti 1983d). Despite this, on subterranean it has caused some particularly severe losses in dry matter and seed production in Western Australia in some situations (Barbetti 1983d, 1989b, 1994; Barbetti and Nichols 1991a, 1995). In particular, total loss of seed production in a cv. Green Range seed crop attacked by rust (Barbetti and Nichols 1991a) indicated that very susceptible varieties are at severe risk in Western Australia. This event, and the potential for severe losses even in partially resistant varieties (Barbetti and Nichols 1991a, 1995), indicated that future varieties must possess adequate rust resistance to prevent major losses occurring from rust.

Powdery Mildew – eastern Australia: Powdery mildew is common on subterranean clover in some areas of New South Wales (Valder 1954; Anon. 1975a). Kable (1967) reported marked differences in susceptibility between pasture legume species with *T. dubium*, *T. glomeratum, T. subterraneum*, and *T. tomentosum*, being the only susceptibility between varieties of subterranean clover with cvs Clare, Mt Barker, Yarloop, Tallarook, and Howard being resistant. While this disease rarely causes any economic loss of production in Victoria, there have been some severe outbreaks in South Australia following early autumn rains and lush pasture growth.

Powdery Mildew – Western Australia: Powdery mildew is also very common on subterranean and other clovers in some areas of Western Australia (Barbetti 1989b). While this disease rarely causes significant economic production losses (Barbetti 1983d), this is not always the case and sometimes disease can be severe. For example, in 1985, the subterranean clover cv. Junee, with resistance to clover scorch was released (Nicholas 1985b), yet several seed production stands of Junee in the south coastal region of Western Australia were severely affected by powdery mildew, indicating that severe disease epidemics could occur in favourable seasons and districts if pure stands of very susceptible varieties were grown. There was renewed interest in ensuring that future clover scorch-resistant varieties were not highly susceptible to powdery mildew as there continue to be some severe outbreaks in Western Australia following early autumn rains and lush pasture growth.

9.3.5 Major fungal pathogens involved and their aetiology and epidemiology

Clover scorch – eastern Australia: Conidia of *K. caulivora* are spread to other leaves and plants by rain splash, animals (Beale 1972) or other mechanical means (Kellock 1971). The conidia can remain viable for at least 18 months on dry seed or dead infected plant residues (Kellock 1971). The disease is carried over long distance in infected hay or seed (Beale 1972; Stovold 1976a). There are significant differences in the severity of the disease induced by different isolates of the clover scorch fungus in eastern Australia (Beale and Thurling 1980a; Chandrashekar and Halloran 1992), and physiological specialisation occurs in different parts of eastern Australia (Helms 1975c; Chandrashekar and Halloran 1992). While it is claimed that moderate temperatures (from about 15 to 25^{o}C), humid conditions and frequent rain with driving winds are most conducive for disease development and spread (Beale 1972), no data were presented to support these claims. The susceptibility of cotyledons and unifoliate leaves decreases rapidly with seedling age (Helms 1975a), while the susceptibility of trifoliate leaves increases to 5 weeks and then decreases (Helms 1975a). Injury also promotes disease development (Helms 1975a). Maximum infection occurs when there is free water on leaves after inoculation and light intensity is relatively low (Helms 1975b). Establishment of infection is favoured by temperatures of 16-24^{o}C, while subsequent disease development is favoured by temperatures of 8-20^{o}C (Helms 1977). The fate of spring pastures is often influenced by the degree to which the disease develops in autumn and winter.

Clover scorch – Western Australia: As in eastern Australia, *K. caulivora* is spread by means of conidia produced in slimy masses in petiole, petiolule, stem and peduncle lesions. There, conidia are mainly spread to other leaves and plants by rain splash (Bokor et al. 1978a,b). There are also significant differences in the severity of the disease induced by different isolates of the clover scorch fungus in Western Australia (Barbetti et al. 1991), induced by different growth stages of the plant (Barbetti et al. 1991), and a study of Barbetti (1995c) was the first confirmation of the existence of distinct races of this pathogen. Bayliss et al. (2001a,b). Bayliss et al. (2001a,b, 2002, 2003) conducted studies to define differences between races 1 and 2 and the infection processes by these two races of *K. caulivora* in subterranean clover and the mechanisms of resistance to this pathogen in subterranean clover. When Anderson et al. (1982) investigated the relationship between environment and clover scorch disease in a subterranean clover pasture in Western Australia, they showed that mean temperatures of 11-17^{O}C and frequent rainfall favoured disease development. However, drought conditions decreased the development and spread of the disease and prolonged warm to hot sunny weather stops disease development. Also in Western Australia it was demonstrated that the susceptibility of plants to this disease is affected by plant age, as with trifoliate leaves susceptibility increases until 5 weeks and then decreases subsequently (Barbetti et al. 1991). If autumn weather is favourably warm, the disease may establish before lower winter temperatures slow further development, and with the return of warmer weather, disease epidemics can rapidly develop in spring.

Cercospora: Cercospora disease, occurs widely on subterranean clover subterranean clover and also on drooping flowered clover (*T. cernuum*) in Western Australia (Barbetti 1983d; Bokor 1983). Few studies have been conducted into Cercospora leaf spot disease on subterranean or other clovers. Barbetti (1985b) showed that mycelial fragments were an effective and reliable inoculum and that the incidence, severity and rate of disease development increased with increasing length of high humidity after inoculation and with increasing concentrations of inoculum. Disease was greatest at 18/13^{O}C followed by 21/16^{O}C and then 15/10^{O}C. *C. zebrina* is well adapted to the Mediterranean-type environment of south- west Western Australia, where it readily over-summers on infested clover residues to release conidia approximately 2 weeks after the first substantial rainfall of the winter growing season, there being a significant relationship between numbers of conidia released with field disease incidence (Barbetti 1987d).

Rust: The fungus survives over the summer on clover debris or on volunteer or irrigated plants and spores are dispersed by wind and rain. Latter (1953) demonstrated the occurrence of two physiological races of subterranean clover rust in Australia, differing markedly in host range and field distribution. Currently, the existence and distribution of races in southern Australia is unknown and needs to be determined such that resistances to different races can be identified and deployed as appropriate.

Powdery mildew: Powdery mildew is spread by windborne spores.

9.3.6 Current Management Strategies – Major fungal disease management using chemicals

Clover scorch – Western Australia: Fungicide sprays are an effective, short term field control measure. In epidemic years up to several tons of fungicide have been applied in Western Australia alone and with up to 9,000 ha of subterranean clover are treated annually with fungicide there for control of clover scorch (Barbetti 1996). The main fungicides used are benomyl or closely related formulations of methyl- 2-benzimidazole carbamate. However, although an economic proposition for subterranean clover- dominant hay and seed production, fungicides are rarely worth using on grazed pastures utilized directly in the field by grazing animals (Bokor et al. 1978b). For maximum benefit, sprays are applied after stock are removed in early spring and repeated 30 days later if necessary (150 to 275 g a.i./ha of a benzimidazole fungicide) (Bokor et al. 1978a,b). High rates of fungicide are used if the disease is well established, but late applications to save stands that are already collapsing are not effective (Barbetti 1993b). As resistant types have replaced susceptible varieties, disease control with fungicides has become less important. However, it could be needed in the future along with appropriate cultural methods if resistance is overcome by changes in the pathogen.

<u>Cercospora – Western Australia</u>*:* This disease can be controlled by the chemicals as with clover scorch. Barbetti (1983a) demonstrated that benomyl sprays in spring reduced leaf infection by up to 88%, petiole infection by up to 59% and increased seed yield by up to 48%. Barbetti (1987b) showed that benomyl and carbendazim gave the best disease control. Smaller disease reductions were obtained with bitertanol, chlorothalonil, propiconazol and thiophanate methyl, while captafol, prochloraz and triadimefon were ineffective. Rates as low as 150 g (a.i.)/ha of benomyl or carbendazim were effective sprayed either in August or in September. Spraying both in August and September gave a better result in only 1 trial, and there was little additional disease control from increasing rate of application of either of the fungicides to 275 g (a.i.)/ha. Disease control from fungicides resulted in seed yield increases up to 68%.

Other diseases: In general, chemical treatments are not justifiable for fungal foliar diseases of annual clovers as they are either largely ineffective and/or the cost simply cannot be justified in terms of the pasture value unless it is a high value hay or seed production stand. Despite this, in fact fungicides can offer substantial yet uneconomic reduction of several foliar diseases of pasture legumes. For example, Barbetti (1986) found that the incidence of *Leptosphaerulina trifolii* and *Phoma medicaginis* was greatly decreased by regular applications of fungicide.

9.3.7 Current Management Strategies – Major fungal disease management using cultural practices

Clover scorch – eastern Australia: The most important cultural control strategy in eastern Australia for controlling clover scorch is close grazing of affected pastures (Beale 1972; Clarke 1980). Cultivations associated with pasture renovation and burning sometimes helped by removing infected residues (Beale 1976), but the benefit was often short lived due to rapid build-up of the pathogen (Beale 1976).

Clover scorch – Western Australia: The most important cultural control strategy in Western Australia for controlling clover scorch is also close grazing of affected pastures (Bokor and Chatel 1973). Grazing removes much of the infected material, thereby reducing the rate of spread of the infection. New growth is consumed before it becomes seriously affected (Bokor et al. 1978a,b). Anderson et al. (1982) found that high stocking rates, such as 10 and 12 sheep/ha, reduced disease incidence by 20-35% compared with 6 sheep/ha. They suggested increased stocking rates as a possible alternative to either chemical control or the replacement of susceptible varieties of subterranean clover in grazed pastures. Cultivations associated with pasture renovation and burning sometimes helped by removing infected residues (Bokor et al. 1978a,b). As resistant types have replaced susceptible varieties, disease control through heavy grazing, burning or cultivation has become less important. However, where existing cultivar resistance is overcome by new races then cultural control strategies are needed until resistant varieties become available and/or are deployed.

__Cercospora__: Unlike clover scorch that can be reduced in severity by close grazing, such cultural practices seem to have a relatively smaller effect on reducing severity and impact of Cercospora (MJ Barbetti, unpubl.).

__Rust__: On susceptible varieties there are no satisfactory control methods but regular grazing of infected pastures may help (MJ Barbetti, unpubl.).

__Powdery Mildew__ On susceptible varieties there are no satisfactory control methods but regular grazing of infected pastures may help (MJ Barbetti, unpubl.).

__Other diseases__: The incidence of several fungi, especially *L. trifolii* and *P. medicaginis*, was greatly increased by the presence of trash from the previous season and residue disposal may assist in reducing severity of several of these diseases (Barbetti 1986). Recently, You et al. (2009) described a new disease observed on *T. dasyurum*, with symptoms beginning as a halo spot and developing into a leaf blight. The causal organism was identified by microscopy and DNA sequence studies as *Botrytis fabae*. This strain of *B. fabae* was also demonstrated to cause disease on foliage of a range of pulse crops, including *Vicia faba*, *Pisum sativum*, and *Lens culinaris*. This study demonstrated the potential of this strain of *B. fabae* to not only pose a significant threat to *T. dasyurum* but also to pulses grown in rotation with *T. dasyurum* that are susceptible to this strain of *B. fabae*.

9.3.8 Current Management Strategies – Major fungal disease management using host resistance

The utilisation of resistant varieties offers the most cost-effective, long-term control measure for fungal foliar diseases of annual clovers, and has been spectacularly successful as the main avenue for the successful control of the most important foliar diseases of annual pasture legumes such as clover scorch disease on subterranean clover.

__Clover scorch – eastern Australia__: Many of the previously common varieties such as Yarloop, Woogenellup, Bacchus Marsh and Seaton Park were highly susceptible to clover scorch (Walker 1956a,b; Kellock 1971; Beale 1972; Beale 1976; Clarke 1980). Historically and continuing today, most research effort has gone into the selection and breeding of clover scorch resistant varieties. Walker (1956a,b) was the first to report different levels of susceptibility to infection between varieties. In *T. subterraneum* ssp. *Subterraneum*. Chandrashekar and Halloran (1990) studied the variation and inheritance of seedling and adult plant resistance to clover scorch in two resistant x susceptible crosses.

In one cross the seedling reaction indicated the presence of a single dominant gene for resistance, while in adult plants, resistance appeared to be conferred by a single recessive gene. In the second cross it appeared that a single recessive gene conferred seedling resistance, but in the adult plant it was conditioned by two recessive genes. Fewer plants were resistant as plant age increased. Beale (1980) showed that screening for seedling resistance under glasshouse conditions did not always relate to results obtained with field sowings, with some lines resistant in the glasshouse being susceptible in the field.

Clover scorch – Western Australia: As in eastern Australia, in Western Australia, many of the previously common varieties such as Yarloop, Woogenellup, Bacchus Marsh and Seaton Park were also found to be highly susceptible to clover scorch (Bokor et al. 1978a,b; Gillespie 1983a). Beale and Goodchild (1980) showed that while the relative levels of resistance of most genotypes of *T. subterraneum* ssp. *yanninicum* were fairly stable over the range of environments studied, interactions between genotype and environment occur, resulting in variation in resistance rankings between sites. Interactions also occur between genotypes and seasons. Beale and Thurling (1980c) demonstrated that clover scorch resistant genotypes of ssp. *yanninicum* are differentiated from the susceptible genotypes by a single major gene, and suggested that the differences in resistance between moderately resistant and susceptible lines were determined by at least four minor genes. In other studies, Beale and Thurling (1980b) showed that dominant alleles were more numerous than recessive alleles among the parents and that gene interactions were relatively unimportant. Much of the previous success in developing subterranean clover varieties with clover scorch resistance was based upon single gene resistance or resistance that behaves as if controlled by a single gene. The collapse of highly resistant varieties in 1990 due to the presence of the new race indicates that continued reliance upon this type of resistance may not be sustainable. The best solution is to breed and use varieties with multiple genes for resistance to the major virulence pathotypes of the pathogen. Failure to do so will lead to vulnerable resistance in released varieties. To accomplish this, the genetic basis of clover scorch resistance to the different races needs to be determined to facilitate the breeding of new resistant varieties. This will enable the development of commercial varieties containing multiple gene resistance to the major virulence genotypes of clover scorch. Barbetti et al. (2005) conducted a study of a partial diallel involving crosses of seven varieties of subterranean clover (*T. subterraneum* ssp. *subterraneum* and ssp. *yanninicum*) to determine the genetic basis of resistance to both race 1 and race 2 of *K. caulivora* at seedling and adult plant stages. Relationships between variance and covariance revealed that adult plant resistance to race 1 and both seedling and adult plant resistance to race 2 were controlled by simple additive and dominant gene effects, whilst seedling resistance to race 1 was controlled by epistatic gene effects. Three of the four most resistant parents (cvs Daliak, Denmark and Meteora) showed a dominant gene effect for adult plant resistance to race 1. However, the other resistant parent, cv. Goulburn, and the most susceptible parent, cv. Woogenellup, both were controlled by recessive gene effects (i.e., gene effects conferred by recessive genes). Variety Denmark again showed dominant gene effects in relation to resistance to race 2 at both seedling and adult plant stages, whilst recessive gene effects were evident in susceptible adult plants of cv. Daliak in response to race 2.

Estimates of genetic variance components confirmed that dominance variance was much greater than additive variance and there were more dominant alleles (i.e., more alleles that had dominant effects) than recessive alleles (i.e., more alleles that had recessive effects) present across all the varieties. An examination of segregation ratios in three F2 populations revealed that inheritance of adult plant resistance to race 1 was controlled by a dominant gene in cv. Daliak and cv. Meteora, and by two dominant genes in cv. Denmark. The additional resistance gene present in cv. Denmark may well explain its apparent stable resistance to both races in the field. This study provides an insight into the genetic basis for and inheritance of resistance to this disease that will assist future breeding programs aimed at improving resistance.

Large scale screening of subterranean clover lines for resistance to clover scorch in Australia started in 1972, and since then more than 13,000 lines have been tested in

Western Australia (Chatel and Francis 1974a, 1974b, 1975, 1977, 1978; Gillespie 1983b; D.J. Gillespie, unpubl.). Many of these were crosses between selections identified earlier in the program as possessing resistance to clover scorch (Gillespie 1983b). Chatel et al. (1973) showed that varieties Daliak and Mt Barker had resistance. Chatel and Francis (1974b, 1975) highlighted the field resistance of Daliak, Toodyay C, and Guildford D, and later the resistance of Daliak was confirmed in glasshouse tests (Chatel and Francis 1974a; Helms 1975c). Chatel and Francis (1977, 1978) found a large number of varieties with useful clover scorch resistance in the field. Beale and Thurling (1979) identified nine ssp. *yanninicum* genotypes with potential for breeding for resistance. By 1983, more than 300 introductions, accessions and crosses had been identified with resistance by the field screening program in Western Australia (Gillespie 1983b). Over the past four decades, many varieties with varying levels of resistance have been released to farmers. For example, Larisa and Trikkala (ssp. *yanninicum*) have useful resistance, while Esperance (ssp. *subterraneum*), also released in 1980, has excellent resistance (Nicholas 1980b,c; Nichols et al. 1996). Meteora, which was released in 1981, is the most scorch resistant of ssp. *yanninicum* even with distinct resistance in the seedling stage (Gillespie 1981). In 1984-85, Enfield, Junee, Green Range and Karridale (ssp. *subterraneum*) were released, all of which have valuable resistance (Hotton 1984; Nicholas 1985a,b). In 1992-93, Denmark, Goulburn (ssp. *subterraneum*) and Gosse (ssp. *yanninicum*) were released, all of which have outstanding resistance (Nichols 1992a,b, 1993). All the above varieties have valuable resistance to clover scorch strains present before the detection of the new race in 1990 (Barbetti 1993a).

In contrast, Izmir is susceptible to race 1 of clover scorch disease and is at least moderately susceptible to race 2 (Nichols et al. 2007b). Barbetti (2007b) undertook separate controlled environment studies to identify the effects of inoculum concentration and the effects of race combinations upon the expression of resistance to clover scorch disease in seedlings of seven subterranean clover varieties. In relation to the percentage of petioles lesioned following inoculation with different conidial concentrations of either race 1 or race 2 of *K. caulivora*, there was a significance effect of races, varieties and of inoculum concentration, and also a significant interaction of races with varieties, and of inoculum concentration with varieties. While levels of disease observed on the varieties tested were generally comparable to those observed in the field, the findings in relation to inoculum concentration may explain why the performance of some subterranean clover varieties in the field in the presence of the clover scorch pathogen can be highly variable, as evidenced by certain incidences of severe disease and/or collapses of field swards of Meteora and/or Karridale in Western Australia. When inoculated with varying proportions of race 1 and race 2, there was a significant effect of race combination treatments, but there was no indication of any additive or interference effects between the two races when present together as a combination on the same plant in relation to host expression of resistance to each individual race. Barbetti (2007b) showed, for the first time, that expression of resistance to *K. caulivora* in subterranean clover is dependent upon pathogen inoculum level but independent of the races present as mixtures. The implications of these studies are three-fold: firstly, that variable expression of resistance in the field is related to inoculum pressure; secondly, that seedling resistance to individual races should be able to be identified even where both races occur together in combination, eliminating the current expensive requirement of utilising a separate field screening site for each race; and, thirdly, that while effective deployment of host resistance is particularly rewarding where cultural and/or chemical strategies are in place to keep the inoculum of the pathogen at manageable levels, it can also be rewarding even under high inoculum pressure.

All new subterranean clover varieties released for use in clover scorch susceptible areas of southern Australia must now possess adequate levels of resistance. Field scores for resistance to *K. caulivora* race 2 have been published by You et al. (2005e) for 30 subterranean clover varieties and 70 breeding lines. Similarly, field scores for resistance to race 1 for 28 varieties and 106 F6-derived breeding lines of subterranean clover have recently been published (Nichols et al. 2008). However, no information on field resistance to *K. caulivora* race 2 has been published for a cohort of subterranean clover mid-season and late flowering breeding lines that are in the final stages of field evaluation across southern Australia, and from which one or more new varieties are likely to be released as new varieties – this needs to be addressed urgently.

Nichols et al. (2010) conducted visual ratings of disease reaction to a mixture of races 1 and 2 of *K. caulivora* on inoculated field plots of 206 accessions of *T. purpureum* (191 var. *purpureum* and 15 var. *pamphyllicum*) collected from the Mediterranean basin and surrounding regions. Disease severity scores of the resistant check, cv. Denmark subterranean clover were clearly differentiated from the susceptible check, cv. Paratta purple clover. Nearly 33 per cent of the accessions were resistant to both races of this pathogen. The results of this experiment have been used to develop a new variety of purple clover with resistance to both races of clover scorch. CPI 139465, a late flowering accession from Turkey, had the lowest disease severity score (1.1) of all accessions. This accession was sorted by Department of Agriculture and Food Western Australia into 9 types, based on leaf markings, which then underwent within-population selection for other characters, including ease of threshing seed from the calyx, high winter and spring vigour and high seed yield (Ewing 2006). This has resulted in the release of the purple clover variety ELECTRA (Nichols et al. 2007c), which Li et al. (2009b) have subsequently confirmed as having resistance to both races of *K. caulivora*.

Li et al. (2009b) tested 12 varieties and 10 F6-derived breeding lines of subterranean clover and two varieties of purple clover (*T. purpureum*) in the field for their response to clover scorch disease caused by race 2 of *K. caulivora* that is restricted in distribution in Western Australia but is generally more virulent than race 1. Two of the varieties (viz. Coolamon subterranean clover and Electra purple clover), and the breeding line SL031 showed outstanding resistance, with disease scores significantly lower than the resistant variety Denmark that had a disease score of 3.3 (0 - 10 scale with 0 = no disease and 10 = death of all plants). A further two varieties (viz. Goulburn, and Karridale) and four of the breeding lines (viz. SL019, SL027, SL029 and SM033) had similar levels of resistance to Denmark. Host responses to race 2 were compared with those against race 1 of *K. caulivora*. Genotypes, such as SL029 and SL031, with combined resistances to both races of *K. caulivora* identified, and their potential for effective management of *K. caulivora* in areas where both races occur was highlighted. This was the first study to show with resistance to race 2 there could provide cross-resistance to race 1 of *K. caulivora* in both subterranean and purple clovers. Nichols et al. (2008) screened 28 varieties and 106 F6-derived breeding lines of subterranean clover were screened in the field for their response to clover scorch disease caused by race 1 of *K. caulivora*. Eleven of the varieties, including Denmark and Goulburn, were classified as resistant. Breeding lines with Denmark parentage had 55% of progeny with resistance, while those of Goulburn had only 19% of resistant progeny, suggesting different modes of inheritance. Selection for resistance to race 2 of *K. caulivora* in the F4 generation markedly increased the probability of selecting F6-derived lines with resistance to race 1, suggesting linkage between genes for resistance to both races.

Cercospora – eastern Australia: No genotype screening has been undertaken in eastern Australia in recent decades against this pathogen.

Cercospora – Western Australia: Barbetti (1985b) demonstrated that while *C. zebrina* can infect all commonly grown Western Australian subterranean clover varieties, Trikkala and Larisa did show some resistance. Of the alternative pasture legumes he artificially screened, lucerne (*Medicago sativa*) medic (*M. littoralis*, *M. truncatula*), dropping-flowered clover (*T. cernuum*), strawberry clover (*T. fragiferum*), rose clover (*T. hirtum*) and white clover (*T. repens*) were all susceptible, but serradella (*O. compressus*) was resistant. Barbetti (1991b) screened 17 commercial varieties of subterranean clover and 10 introduced lines for resistance to cercospora disease under a controlled environment, and responses were compared with their performance in the field. There were large differences between varieties in the severity of Cercospora disease under both conditions and there was, overall, good correlation between data from the field and those from the controlled environment for the different parameters. In that study, Daliak, Dalkeith, Esperance, Mt Barker, Nungarin and Rosedale were highly susceptible to Cercospora disease under both controlled environment and field conditions, while Clare, Larisa, Meteora and Yarloop were assessed as having a high degree of resistance under both conditions. Although variation in resistance to Cercospora disease has been demonstrated under controlled conditions (Barbetti 1985b, 1991b) and in the field (Barbetti 1991b; Barbetti and Nichols 1994), Barbetti (1991b) clearly advocated that all potential new varieties and parental materials are best screened for Cercospora resistance under field conditions as this gives the most reliable indication of commercial field performance. Barbetti and Nichols (2005a) screened 96 genotypes, including 14 varieties, of *T. subterraneum* var. *subterraneum* and var. *yanninicum* in the field for resistance to Cercospora. Seven genotypes, *viz*. 84S43-13, 84S43- 15, EP132Sub-E, 84Y32-59, 83Y83-23, 84Y32-42 and 83Y79-20, were totally resistant to Cercospora. A further 26 genotypes, including the varieties Meteora and Napier, had an incidence of Cercospora of <1 (0-10 scale) and no consequent leaf collapse from the disease (score 0; 0-10 scale), while 15 genotypes had incidence scores between 1 and 2 without any leaf collapse evident. There was excellent overall correlation between Cercospora incidence and leaf collapse across the genotypes tested, with both strong quadratic ($y = -0.17x^2 + 2.50x + 1.33$; $R^2 = 0.89$) and linear ($y = 0.96x + 1.63$; $R^2 = 0.82$) components to this relationship. There was circumstantial evidence of ecogeographical differences for Cercospora resistance among ecotypes collected from different regions. Out of 8 overseas introductions with Cercospora incidence scores of <2 and a Cercospora leaf collapse score of 0, six were from Sardinia and one each were from Portugal and Greece. In contrast, all seven Sicilian ecotypes had Cercospora incidence scores of 7.7 or greater. The high degree of resistance observed in many of the genotypes to Cercospora highlights the existence of many excellent sources of resistance that could and should be exploited in breeding and development programs to minimise production losses in Australian subterranean clover pastures. There is increased susceptibility of subterranean clover to *C. zebrina* when K levels in soils are significantly below or above ‘normal’ (J. Dempster, M. You, M.J. Barbetti and K. Sivasithamparam, unpubl.).

Rust – eastern Australia: As early as the 1940's Loftus Hills (1942, 1944) tested the reaction of subterranean clover varieties to leaf rust. He demonstrated varietal reactions ranged from highly susceptible to highly resistant (Loftus Hills 1942), that early maturing types in particular were not attacked (Loftus Hills 1944) and that disease resistance is an inherited character (Loftus Hills 1944).

Rust – Western Australia: Barbetti and Nichols (1991c, 1994) demonstrated that varietal reactions to rust ranged from highly susceptible to highly resistant. Barbetti and Nichols (1991c) advocated the necessity for testing rust resistance under field conditions if host reactions were to provide a reliable indication of field. Barbetti and Nichols (2005b) screened 57 genotypes, including 10 varieties of *T. subterraneum* var. *subterraneum* and var. *yanninicum* in the field for resistance to rust using artificial inoculation. There was outstanding resistance among the var. *yanninicum* types with all but 1 genotype showing no rust symptoms. Several var. *subterraneum* genotypes also showed only a low rust incidence (<3.5 on a 0–10 scale) with little or no leaf collapse from rust infection, including 83S19–07, CPI 103906F, EP132Sub-E, 84S20–02, and 84S20–01. Several other lines had a significant incidence of rust, while little leaf collapse from the disease was evident. Several highly susceptible lines were identified, including varieties Green Range, Seaton Park and York, all with 100% of leaves affected by rust and extensive leaf collapse. There was excellent positive correlation between rust incidence and leaf collapse across the genotypes tested ($R^2 = 0.91$). The excellent rust resistance observed in the majority of var. *yanninicum* lines and the good resistance in some var. *subterraneum* lines, indicates that these are useful sources of resistance that can be exploited, either directly as new varieties to minimise leaf collapse from this disease or as parents in breeding programs to develop more rust-resistant varieties.

Powdery Mildew – eastern Australia: Kable (1967) reported marked differences in susceptibility between varieties of subterranean clover.

Powdery mildew – Western Australia: Barbetti and Nichols (1991d) screened 33 subterranean clover varieties, comprising 16 commercial varieties and 17 promising midseason breeding and introduced lines, for resistance to powdery mildew under controlled environment conditions, and where possible, comparisons were made with their performance in field plots. There were large differences between varieties in the incidence and severity of powdery mildew on plants. Under controlled environment conditions, Tallarook, CPI 47308C, CPI 89860D, 75S13-12, CPI 6.53284 CPI 89820D and Clare had the lowest levels of leaf infection, the lowest mildew sporulation scores and the least amount of leaf scorching from mildew infection. Karridale had the highest level of leaf infection, followed by 69837-1, 76841-1, Junee, Bacchus Marsh and 69S37-3. Under field conditions, Tallarook, CPI 47308C, CPI 65328A and CPI 65328F were mildew-free, while CPI 89777C, CPI 89860D, CPI 89830F, CPI 89841E, Clare and Rosedale had good resistance. Junee was clearly the most susceptible under field conditions, followed by 76S41-1, 69837-3, Karridale, Bacchus Marsh and 69S37-1. There was generally excellent correlation between the different powdery mildew disease parameters, namely leaf infection, mildew sporulation and leaf scorch, measured under controlled environment conditions, and there was also good overall correlation between controlled environment and field data. Bacchus Marsh, Junee, 69337-1, 69837-3 and 76341-1 were highly susceptible under both controlled environment and field conditions, indicating that either environment could be used to identify highly susceptible varieties. Under controlled environment conditions, a high degree of resistance was observed in Clare, CPI 47308C, CPI 65328A, CPI 89820D, CPI 89860D and 75S13-12, while field plots of the varieties Tallarook, CPI 47308C and CPI 65328A showed a complete absence of powdery mildew. These varieties will have value as parents in breeding programs for powdery mildew resistance.

9.3.9 Current Management Strategies - Integrated fungal disease management

Integrated disease management strategies are widely and successfully utilised for management of some fungal foliar diseases on pasture legumes. For example, such as for control of *Kabatiella* on *Trifolium* species across southern Australia (Barbetti 1996), where the application of fungicides in conjunction with using partially resistant varieties and/or application of close grazing provides more benefit than the same or greater amounts of fungicides applied to susceptible varieties, especially where grazing is minimal (Barbetti 1996). Further integration and deployment of integrated control strategies will remain a key focus until varieties of annual clovers with improved host resistance to all major foliar diseases are available, and, even then, will remain important should such host resistance succumb to more virulent races of one or more fungal foliar pathogens that affect annual clovers.

9.4 Viral diseases

The textbook 'Virus diseases of Plants in Australia' by Buchen-Osmond et al. (1988) lists all viral pathogens then known to infect annual pasture clover species in Australia. Virus diseases of annual pasture clover species were included in several early general reviews (Johnstone and Barbetti 1987; Panetta et al. 1993; Jones 1996). Johnstone and MacLean (1986) specifically reviewed virus diseases of subterranean clover. Seed-borne viral diseases of subterranean clover were major components of more recent reviews (Jones 2000, 2004a).

As a relatively small number of the viruses are or overriding importance on annual clovers, other viral pathogens that can sometimes be important on them will not be discussed in detail but are addressed briefly below.

9.4.1 Minor viruses

Alfalfa mosaic virus (AMV). AMV is recorded infecting subterranean clover plants in TAS, WA, NSW, VIC and SA (Johnstone 1987; Johnstone and McLean 1987; Buchen-Osmond et al. 1998; Jones and McKirdy 1990; Jones 1992, 1996; McKirdy and Jones 1994). Infected plants show no symptoms, or, with some varieties, characteristic line patterns in leaves and some dwarfing. The virus is non-persistently aphid-transmitted and seed-borne (Jones 1992). In infected spaced subterranean clover plants growing in plots, AMV decreased herbage dry weight by 20-49% and seed yield by 71% and was seed-borne (2%) in this species (Jones 1992; McKirdy and Jones 1994). Francki et al. (1983) reported frequent infection of subterranean clover in SA. Helms et al. (1993) reported that in 1984-1985 AMV often occurred in subterranean clover pastures in TAS, VIC, SA, WA and NSW. However, in a subsequent survey of 94 subterranean clover pastures in WA in 1993 which included some of the same pastures, McKirdy and Jones (1995) did not detect it in random samples. They suggested that the AMV results of Helms et al. (1993) might partly represent 'false positives' arising from the way their tests were done (see Muller et al. 1993). Johnstone and McLean (1987) suggested that subterranean clover pastures are likely to be at risk from AMV when grown in close proximity to lucerne or white clover pastures as AMV occurs commonly in them. However, this is unlikely to occur in WA where neither of these two pasture species are widely grown. Further surveys in eastern Australia and Tasmania are needed to clarify how widespread AMV is in subterranean clover pastures distant from lucerne or white clover pastures. Latham and Jones (2001) reported AMV infection in plots of the following alternative annual clovers in WA: arrowleaf clover, balansa clover, bladder clover, cupped clover, crimson clover, eastern star clover, gland clover, Moroccan clover, helmet clover, Persian clover, purple clover, rose clover, and sea clover.

Beet western yellows virus (BWYV). BWYV is recorded infecting subterranean clover at several locations in TAS (Johnstone and Duffus, 1984), and was confirmed as the cause of leaf reddening in subterranean clover in WA (Johnstone and McLean 1987; Buchen-Osmond et al. 1998). The symptoms of BWYV in subterranean clover consist of leaf reddening which first appears near the middle of the leaflets, then spreads radially, always being more intense on the lower leaf surface. These symptoms contrast with those of *Subterranean clover red leaf virus* (SCRLV) which are generally more severe, develop from the margins of the leaflets and are most obvious on the upper leaf surface (Johnstone and McLean 1987). BYWV has not been reported infecting other annual clover species in Australia. However, since this virus is one of the most widespread viruses in southern Australia infecting a wide range of wild and crop legume and other hosts in all states (e.g. Coutts and Jones 2000; Jones 2004b; Coutts et al. 2006; Salam et al. 2011), it may be widespread in annual clover pastures causing symptoms likely to be confused with those of SCRLV or nutritional disorders. Thus, its economic importance in annual clover pastures may be greater than currently understood.

Clover yellow vein virus (CYVV). CYVV is recorded infecting subterranean clover in TAS, NSW and VIC (Johnstone and McLean 1987; Buchen-Osmond et al. 1998). It generally causes a mosaic and vein clearing in subterranean clover but some isolates from Victoria cause lethal necrotic reactions (Johnstone and McLean 1987). Helms et al. (1993) reported that in 1984-1985 CYVV often occurred in subterranean clover pastures in TAS, VIC, SA, WA and NSW. However, in a subsequent survey which included some of the same pastures, McKirdy and Jones (1995) did not detect it in random samples from subterranean clover pastures in WA and suggested that the CYVV results of Helms et al. (1993) might represent ‘false positives’ arising from the way their tests were done (see Muller et al. 1993). It has not been reported infecting other annual clover species in Australia. This virus is most likely to cause problems in annual clover pastures when they are grown near to pastures of its principal hosts, white clover and lucerne.

Lucerne transient streak virus (LTSV). LSTV is recorded infecting crimson clover in VIC (Buchen- Osmond et al. 1998). This virus is likely to spread to annual clover pastures when they are grown near its principal host, lucerne. It seems of little economic significance in annual clover pastures.

White clover mosaic virus (WCMV). WCMV is recorded infecting subterranean clover in TAS and ACT (Buchen-Osmond et al. 1998). Infected subterranean clover plants display a mild mottle (Johnstone and McLean 1987). This virus is only ever likely to cause problems in annual clover pastures when they are grown near to pastures of its principal host, white clover.

9.4.2 Disease symptoms – Important viral diseases

Subterranean clover mottle virus (SCMoV): The first symptoms of SCMoV infection in subterranean clover plants are vein clearing (yellowing of minor veins) in newly expanded leaves, followed by a distinct mottle in younger leaves. Leaves are often misshapen and small. As plants age, they become dwarfed and stumpy in appearance through a shortening and thickening of stems. Some varieties, such as Junee and Seaton Park, may show reddening in newly infected leaves, especially in winter when plants are growing slowly. The disease is most noticeable in spring, when the number of infected plants increases rapidly and newly infected plants develop more distinct disease symptoms (Wroth and Jones 1991, 1992a,b; Ferris and Jones 1994). Within infected pastures, inspection of heavily trampled areas near gates or water troughs, and along tracks made by livestock or the wheels of vehicles often reveals a greater concentration of symptom-affected plants than elsewhere. This spatial pattern of distribution is consistent with spread by contact transmission rather than by a specific vector (Wroth and Jones, 1991 1992a,b; Helms et al. 1993; Ferris and Jones, 1994; Jones et al., 2001).

Subterranean clover varieties differ in susceptibility to SCMoV. Sub sp. *subterraneum* varieties were either susceptible or partially resistant and sub sp. *brachycalycinum* varieties had either partial resistance or strong resistance, but all sub sp. yanninicum varieties had strong resistance (Wroth and Jones 1992a; Ferris et al. 1996).

In addition to subterranean clover, SCMoV has also been found naturally infecting arrowleaf clover, and a few wild annual clovers, such as cluster clover (Francki *et al*., 1983, 1985; Ferris and Jones, 1994). When infected, the other clovers develop similar symptoms to those of infected subterranean clover. However, it is likely that other naturalized and cultivated clovers become infected, because berseem, drooping-flowered, crimson, shaftal, woolly, purple, rose and hop clovers were readily infected in glasshouse studies. By contrast, suckling and balansa clovers were resistant to infection in the glasshouse and when growing in subterranean clover pastures heavily infected with the virus (Wroth and Jones, 1991, 1992a,b; Ferris and Jones, 1994; Ferris et al. Jones et al., 2001).

In the field, subterranean clover plants infected with this virus exhibit symptoms similar to those induced by BYMV (Francki et al., 1985; Ferris and Jones 1994). However, a concentration of infected plants in tracks made by stock or vehicle wheels and in heavily trampled areas distinguishes SCMoV from the other virus (Ferris and Jones, 1994, 1995; Jones 1996; McKirdy et al. 1998; Jones et al. 2001).

<u>Bean yellow mosaic virus</u> <u>(BYMV):</u> Diseased subterranean clover plants develop obvious initial leaflet vein clearing, followed by mottle, leaf distortion and plant dwarfing. The first symptoms of infection are vein clearing, followed by distinct yellowing between the veins, mottle and leaf deformation. Later, the plants become dwarfed. The severity of the dwarfing varies with variety, e.g. Esperance is more severely affected than Karridale or Green Range. BYMV disease incidence increases rapidly in spring, but infected plants may sometimes be seen in autumn and winter (Wroth and Jones 1991; Ferris and Jones,1994).

Subterranean clover varieties differ in the extent to which they become infected with BYMV in the field, most plants becoming infected with late maturing varieties and fewest plants becoming infected with early flowering varieties (Aitken and Grieve 1943; Ferris and Jones 1996). Cv. Rosedale was an exception as few plants became infected despite its intermediate maturity. When it was aphid-inoculated with seven different BYMV isolates, cv. Rosedale showed strain-specific systemic hypersensitive resistance (Ferris and Jones 1996). Hutton and Peak (1954) reported finding systemic hypersensitivity to a potyvirus in cv. Dwalganup, and in selections ‘Northam First Early’ and ‘Pink Flowered’. However, Ferris and Jones (1996) did not confirm this using the same seven BYMV isolates they used to test cv. Rosedale. Possibly, Hutton and Peak (1954) were working with the closely related virus CYVV instead of BYMV.

Alternative annual pasture and naturalized clovers (including alsike clover, annual strawberry clover, arrowleaf clover, balansa clover, bladder clover, cluster clover, cupped clover, drooping flowered clover, gland clover, hares' foot clover, helmet clover, hop clover, hop ligurian clover, narrow-leaf clover, Moroccan clover, Persian clover, purple clover, rose clover, rough clover, sea clover, suckling clover, suffocated clover, and woolly clover) also become infected with BYMV naturally in Australia. Hares' foot clover develops systemic necrosis, but the others develop leaf mottle and/or leaf deformation with or without plant stunting. Severity of BYMV symptoms varies widely between species with some (e.g. bladder clover, cupped clover and hares' foot clover) displaying the very severe symptoms and others developing very mild symptoms (e.g. drooping flowered clover, rough clover and suffocated clover). The different species also vary widely in susceptibility to BYMV infection by aphids with some becoming infected readily (e.g. suckling clover and hop clover) and others only rarely (e.g. balansa clover, drooping flowered clover, hares' foot clover, rough clover and suffocated clover). The high susceptibility and sensitivity to BYMV in some alternative annual pasture and forage clovers is cause for concern. This is especially so when they are intended for sowing in BYMV-prone high rainfall zones (McKirdy and Jones 1995; McKirdy et al. 2000).

<u>*Cucumber mosaic virus* (CMV):</u> CMV causes symptoms of leaf mottle, leaflet downcurling and plant stunting in subterranean clover plants, but symptom severity varies with variety and virus strain. The leaflet downcurling symptom is highly characteristic and helps to distinguish CMV symptoms in subterranean clover from those caused by other viruses. Varieties of subterranean clover differ in their susceptibility to CMV, cvs Enfield, Green Range, Nangeela and Yarloop being very susceptible while others such as Dwalganup, Larissa and Uniwager are slow to become infected (Johnstone and McLean 1987; Jones and McKirdy 1990). The disease incidence increases rapidly in spring in plots of susceptible varieties, but infected plants may sometimes be seen in autumn and winter (McKirdy and Jones 1990).

When McKirdy and Jones (1994b) and Latham et al. (2001) exposed annual pasture and naturalised wild clover species to natural infection with CMV, arrowleaf clover, balansa clover, bladder clover, cluster clover, crimson clover, cupped clover, drooping flowered clover, eastern star clover, gland clover, hares' foot clover, helmet clover, Moroccan clover, Persian clover, purple clover, sea clover and suckling clover, all became infected. The commonest symptoms were leaf deformation and plant stunting but mottle, leaf deformation and symptomless infection also occurred. Severity of symptoms varied widely with bladder clover and Moroccan clover developing exceptionally severe symptoms but cupped clover, eastern star clover and purple clover developing symptomless infection. Crimson clover was the most susceptible to infection but sea clover and bladder clover were relatively resistant.

<u>*Subterranean clover stunt virus* (SCSV):</u> Uninfected leaves formed prior to systemic infection of SCSV- affected plants develop reddish tints while those formed later have very small leaflets and show yellowing and clearing of veins, particularly at their margins and at leaflet apices. Marginal chlorosis, twisting and cupping of leaves also develops, along with proliferation of axillary shoots due to breakdown of apical dominance. Internode length is drastically shortened and petiole length reduced. Infected plants are stunted. Naturally infected drooping flowered clover develops similar symptoms (Smith 1966; Grylls 1972; Johnstone and McLean 1987; Buchen-Osmond et al. 1988).

Subterranean clover red leaf virus (SCRLV): The older leaves of sensitive subterranean clover varieties show intense leaflet reddening which develops progressively from the leaflet margins (Kellock 1971; Johnstone and MacLean 1987). Expression of bright leaf reddening caused by SCRLV in lower leaves of subterranean clover plants was greatly inhibited by low (15^{o}C) or high (33^{o}C) temperatures when the symptoms were reddish-brown or brown. Leaf reddening developed most readily at 20-25^{o}C (Helms et al. 1984). Its symptoms in subterranean clover are often confused with those of lower leaf reddening arising from nutrient deficiencies or other stressors. This often results in its incidence being over estimated in subterranean clover when based on symptoms alone.

9.4.3 Extent and impact of viral diseases on pasture productivity

Diminished feed for stock caused by widespread infection with SCMoV and BYMV in annual clover pasture is estimated to cause economic losses of A$31 million per year to the Australian dairy industry. These two viruses also cause substantial annual economic losses to the Australian wool and meat industries (Ferris and Jones, 1994, 1995; Jones, 1996). Jones (1996, 2004a) summarised the research then available on the extent and impact of virus diseases on subterranean clover pasture production.

SCMoV: SCMoV was originally discovered in 1979 in plots of subterranean clover cv. Dinninup at Karridale in south-west Australia (McLean 1983; McLean and Price 1984). It was first described by Francki et al. (1983). It causes the commonest and most damaging virus disease of annual pastures in southern Australia. It occurs in TAS, WA, SA, VIC, and NSW. The incidence of infection often reaches very high levels in subterranean clover plants within old pastures (Johnstone and McLean, 1987; Wroth and Jones, 1992b; Helms *et al*., 1993a). In national surveys in 1984-1986, Helms et al. (1993a) recorded SCMoV incidences of up to 93% in individual pastures in WA, and this virus was the most prevalent of the viruses tested for. Individual pasture incidences were not provided for SCMoV in TAS, SA, VIC or NSW, but the virus was detected frequently in each state. In WA, surveys in 1989-1990 detected SCMoV in 61% of pastures sampled at incidences up to 50%. It was commoner in pastures >5 years old than in ones sown more recently and final infection incidences were greatest in regions with high rainfall where pasture growth was lush (Wroth and Jones 1992b).

No virus surveys have been undertaken in Australia to establish the occurrence of SCMoV in commercial pastures of alternative annual clovers. No SCMoV was detected in breeding, evaluation or seed- increase plots of these species (e.g. Jones 1999b), but this is only to be expected as grazing and mowing were not used so the virus would be unlikely to spread in them even in highly SCMoV susceptible species.

The dwarfing that infection with SCMoV causes in subterranean clover plants within pastures greatly decreases herbage and seed production. In spaced plants of susceptible subterranean clover varieties, SCMoV decreased herbage dry weight by 81-92% and seed yield by 90% (Wroth and Jones (1992b). In field experiments with grazed monoculture swards of subterranean clover, Ferris and Jones (1995) found herbage yield (dry weight) losses of up to 44% and seed yield losses of up to 43%. A linear relationship existed between increasing losses in herbage yield (dry wt) and increasing percentage incidence of infected plants (Ferris and Jones 1995; Jones 2006). Seeds from infected plants were smaller and produced less vigorous seedlings. This together with seed yield losses diminishes feed for stock and depletes the clover seed bank in the soil which reduces the ability of pastures to regenerate from seed each year (Wroth and Jones, 1992b; Ferris and Jones, 1994; 1995; Jones 1996; Barbetti et al. 1996; Barbetti and Jones 1999). Over the years, cumulative annual seed yield losses reduce the clover component within virus-infected pastures, which gradually become unproductive, as grasses and weeds increasingly dominate them (Ferris and Jones, 1994, 1995; Barbetti et al., 1996; Jones 1996; Barbetti and Jones, 1999). Thus, SCMoV is a major contributor to the decline of the subterranean clover component within mixed species, self-regenerating annual pastures.

<u>BYMV</u>: In Australia, BYMV was first reported infecting subterranean clover in WA in 1941 (Norris, 1943). BYMV/CYVV was subsequently found infecting subterranean clover in all of the southern states of eastern Australia and in TAS (Aitken and Grieve 1943; White 1945; Watson 1949, Hutton and Peak 1954; Harvey 1956; Johnstone and McLean, 1987; Freeman and Aftab 2011). Based mainly on pasture inspections in 1987-1998, Jones and McKirdy (1990) reported that BYMV was common WA. In 1989-92, surveys of subterranean clover pastures in WA found that BYMV incidences varied from year to year and that final incidences were greatest in districts with high rainfall (McKirdy et al. 1994. In the 1992 epidemic year they were up to 90% within individual pastures but in 1990 they did not exceed 64% (McKirdy et al. 1994). No detailed surveys to establish BYMV incidence in pastures in eastern Australia have been undertaken as yet but observations indicate similar BYMV infection incidences to those in WA (Johnstone and McLean 1987; Jones 1996).

In 1994-1998, BYMV commonly infected plants growing in plots subterranean clover and alternative annual clovers at breeding, seed increase and evaluation sites in WA (McKirdy and Jones 1988a; McKirdy et al. 1988a; Jones 1999a). The most frequently infected species were arrowleaf clover and crimson clover, but balansa clover, bladder clover, Moroccan clover and *Trifolium pallidum* were also infected at more than one site. Species found infected at one site only included berseem clover, hares' foot clover, annual strawberry clover, helmet clover, cluster clover, rose clover, ball clover, purple clover, and Persian clover (Jones 1999b). BYMV incidences of 95-100% were recorded at Narrikup in 1998 in annual strawberry clover, balansa clover, ball clover, berseem clover, crimson clover, *T. pallidum* and Persian clover (Jones 1999b). These results suggest that BYMV may pose a major problem in commercial pastures of these species throughout Australia. However, no virus surveys have been undertaken in commercial pastures of alternative annual clovers.

In early studies with spaced plants of five subterranean clover varieties, Hutton and Peak (1954) reported BYMV/CYVV-induced growth reductions of 26-77%, and the infected plants often failed to set seed. In later studies with spaced plants of subterranean clover, a mild isolate of BYMV decreased herbage and root dry weights by 31-40%, but a severe BYMV isolate decreased them by 60-63% in the same variety and by 79-80% in another variety. The severe isolate caused seed yield losses of 58-76% (Jones 1992). In six field experiments in 1989-1992, swards of six subterranean clover varieties were infected with BYMV by transplanting infector plants of subterranean clover into them and allowing natural spread by aphids. The mown swards were grazed by sheep or mown to simulate grazing (Jones 1994). In partially infected swards of cvs Green range, Karridale and Leura, BYMV decreased overall herbage dry weight by 12-16% and seed yield by 37-38%. In grazed swards of cvs Esperance and Karridale, BYMV decreased overall herbage dry weight by 18-39% and seed yield by 11-12%, but these swards were only partially infected and herbage yield losses within individual symptom-affected patches were 28-49%. In another experiment with grazed swards of cvs Junee and Karridale, BYMV-induced losses within symptom-affected patches were 21-29% for herbage and 15-25% for seed. In a further grazing experiment with cvs Denmark and Leura, yield losses within symptom-affected patches were 18- 25% for herbage and 35-47% for seed. Seed yield losses were due to development of smaller and fewer seeds. As with SCMoV, the dwarfing BYMV causes in subterranean clover within pastures decreases the amount of feed available, and the relationship between increasing losses in clover herbage yield in swards (grazed or mown) and increasing incidence of infected clover plants is linear (Jones 1994, 2006). Thus, BYMV infection of subterranean clover pastures is cause for concern, not only as regards reduction in available feed but also as regards depletion of the seed bank, which when compounded year by year, results in pasture deterioration. Early and prolonged aphid activity, heavy grazing and extended growing seasons are all likely to magnify the problem (Jones 1994).

Studies overseas investigated herbage and seed losses caused by BYMV in red clover and arrowleaf clover. In red clover BYMV reduced forage and seed yields, and winter hardiness. However, with arrowleaf clover the experiments in which BYMV reduced yields involved plants in control environment chambers rather than field evaluation (reviewed by Barnett and Diachun 1986).

<u>CMV</u>: Contamination of seed stocks with CMV is frequent in subterranean clover breeding and selection programmes (Jones and McKirdy 1990). Such contamination is important as it leads to release of seed stocks of new varieties that are already infected before they are grown commercially. Farmers may then suffer financial losses from unknowingly sowing virus-contaminated seed, which, in turn, can have potentially serious ‘duty of care’ legal implications for the unwary plant breeder or selector (Jones 1991; Jones 2004a). However, no CMV was detected in 17 commercial subterranean clover seed stocks from 1986 or in a survey of 1987 commercial subterranean clover pastures in which BYMV was often found (Jones and McKirdy 1990). CMV was detected in plots of Persian clover and balansa clover in 1987 at Many Peaks in WA (Jones and McKirdy 1990), and in arrowleaf clover, crimson clover and Moroccan clover in WA in 1996 (McKirdy S.J and Jones R.A.C, unpubl.), but surveys to determine its incidence in commercial alternative annual clover pastures have never been undertaken in Australia.

In pot tests, CMV decreased herbage production by 60% in subterranean clover plants (Garrett 1987), and herbage and root dry weights of subterranean clover by 49% and 59%, respectively (Jones and McKirdy 1990). In spaced subterranean clover plants, CMV decreased herbage dry weight by 59-63% and seed yield by 45%. In rows sown with infected seed in which the virus was spread naturally by aphids reaching 75% (cv. Green Range) or 44% of plants (cv. Esperance), losses in herbage production of 42% and 29% were recorded (Jones and McKirdy 1990). In field experiments in 1988-89 which examined the effects of sowing seed stocks of different varieties without or with 1-7% CMV seed infection and the resulting swards were mown or left undefoliated, the rate of virus spread by naturally occurring aphids was faster and final CMV incidences greatest when the swards contained more seed-infected plants (Jones 1991). In two initial experiments in which CMV spread was extensive, the herbage dry weight yields were 12-30% (undefoliated) and 17-24% (mown), and seed yield losses were 53-64% (undefoliated). When CMV spread was extensive in three further experiments, herbage yield losses of 25-28% were recorded inside infected patches of mown or undefoliated swards, and seed yield losses were 40-42% (mown) and 53% (undefoliated). When CMV spread was small, the herbage and seed yield losses it caused were lower (Jones 1991). This research demonstrates that sowing CMV-infected seed lots has serious consequences for subterranean clover pastures in the year of sowing. However, when the incidence of CMV infection in self regenerating grazed pasture swards of three varieties sown with 1% CMV-infected seed was followed over 7 years after the initial sowing, infection did not persist longer than 5 years (Jones 1991; McKirdy and Jones 1994b). This suggests that CMV is may not cause significant losses in older pastures.

SCSV: More than 70% infection incidences with SCSV have been recorded in VIC pastures (Smith 1974) and 10-30% incidences were common in NSW in the 1950s. Incidences sometimes exceeded 75% causing serious fodder losses (Grylls and Butler 1959). Subterranean clover pastures sometimes failed in south-east NSW due to severe dual infections with SCSV and potyviruses (BYMV and/or CYVV) (Grylls and Peak 1969). However, SCSV may have become less important since then in NSW (Stovold 1983a). In pot tests, the growth of plants of subterranean clover varieties was reduced by about 60% (Johnstone 1983). SCSV induced losses have not been quatified in grazed swards.

SCRLV: Johnstone and McLean (1987) suggested that subterranean clover pastures are most likely to be at risk from SCRLV when grown in close proximity to perennial pastures of lucerne, red clover or white clover as it occurs commonly in them. Also, diseased plants occurred most commonly close to such reservoirs of infection, especially white clover. However, this is unlikely to occur in WA where neither of these two pasture species are widely grown. Further surveys in eastern Australia and Tasmania are needed to clarify how widespread SCRLV is in subterranean clover pastures distant from lucerne of white clover pastures. Helms et al. (1993) reported that in 1984-1985 SCRLV often occurred in subterranean clover pastures in TAS, VIC, SA, WA and NSW. However, in a subsequent survey of 94 subterranean clover pastures in WA in 1993 which included some of the same pastures, McKirdy and Jones (1995) did not detect it in any random samples. As for AMV and CYVV (see above), they and suggested that the SCRLV results of Helms et al. (1993) might partly represent ‘false positives’ arising from the way their tests were done (see Muller et al. 1993).

SCRLV reduced fresh weight of SCRLV-infected plants 2-3-fold (Helms et al. 1984). It reduced the growth of 17 subterranean clover varieties by 61% in TAS (Johnstone 1983). In TAS, establishment failures occur sporadically when more than 90% of emerging seedlings become infected with SCRLV. Extensive infection in spring caused subterranean clover pastures to collapse before they could be cut for hay or silage (Anon. 1986b; Kellock 1971). Hay production was reduced by more than 50% (Smith 1974). When incidences of SCRLV infection are low, the diseased plants soon disappear from pasture due to interplant competition and increased susceptibility to fungal pathogens, leading to grass dominant pastures. SCRLV infection also enhances accumulation of oestrogens in subterranean clover (Johnstone and Barbetti 1987). SCRLV induced losses have not been quatified in grazed swards.

9.4.4 Viral pathogens involved and their aetiology and epidemiology

SCMoV: Early studies showed SCMoV to be particularly stable, reaching high concentrations within infected plants (Wroth and Jones 1992*a*). Infectivity survived drying for 4 days on a metal surface and when mixed with bovine saliva and kept at 20^{o}C for 4 weeks (McKirdy et al. 1998). Such stability and high concentration are typical of contact-transmitted viruses. Transmission occurs through leaf and stem wounds caused by grazing and trampling by stock, and by mowing for hay production or to prevent swards from becoming rank. Simulated grazing and trampling experiments in pots in the glasshouse and small swards, and mowing experiments showed that trampling was the most efficient method of transmission.

Later observations confirmed that SCMoV spreads readily when clover plants are crushed under vehicle wheels. Modern pasture management systems minimise wastage of available feed by using intermittent short bursts of heavy grazing and these enhance spread of SCMoV. SCMoV is seed- borne in annual clover species but no evidence of transmission by a specific vector was found. Infection is introduced to new, healthy pastures by sowing SCMoV-infected seed or contact transmission when moving livestock, mowers, other farm machinery or vehicles into them from nearby infected pastures (Wroth and Jones 1992a,b; Ferris and Jones 1994, 1995; Jones 1996, 2004a; McKirdy et al. 1998; Jones et al. 2001).

Where SCMoV occurs, the annual clover species and pastures involved are annually self-regenerating. Under typical Mediterranean-type climatic conditions, as in much of southern Australia, subterranean clover seeds germinate following the first rains in late autumn. The clover plants grow over winter and produce more seeds before dying from lack of moisture in spring. Subterranean clover buries its seeds, hence its name, and they survive dormant in the soil over the dry summer period when the pasture is dead. The virus is only seed-borne at low levels, with 0.5% the maximum rate of transmission to seedlings recorded (Wroth and Jones 1992a; Njeru et al. 1997). Nevertheless, such low transmission rates can lead to numerous infected seedlings/ha within pastures. Following germination of pastures in autumn, the primary virus infection sources within them are clover plants infected from these seeds. Subsequent spread by contact transmission is polycyclic and occurs from these primary foci, resulting in expanding patches of infection that surround them and new infections further away consisting of isolated plants or patches that are most evident in heavily trampled areas. If spread starts early and the stocking rate is high, further cycles of spread accelerate the rate of increase such that virtually all susceptible plants may be SCMoV-infected before plants die from lack of moisture at the end of the growing period. Thus, the earlier virus spread starts the greater the final incidence reached (Wroth and Jones 1992b; Ferris and Jones 1994, 1995; Jones 1996, 2004a; McKirdy et al. 1998; Jones et al. 2001).

Knowledge that SCMoV has a narrow host range, is seed-borne, is spread readily by contact without the need for a vector, of its temporal and spatial patterns of spread and of how SCMoV-resistant clover varieties behave in the field is critical information required to understand how epidemics develop (Jones 2004a). The magnitude of the primary infection source is influenced not only by the amount of infected seed in the clover seed bank, but also by the extent of successful establishment of seed-infected versus healthy seedlings. Greater rainfall before the growing season starts results in high soil moisture contents during early growth of pastures. This favours survival of the less vigorous clover seedlings infected with SCMoV *via* seed. In contrast, dry conditions during early growth diminish survival of infected seedlings. SCMoV-infected clover plants become overshadowed and eventually killed by neighbouring healthy plants when grazing pressure is low or absent. When new growth is removed by heavy grazing, however, they remain available to act as sources for further spread (Ferris and Jones 1994, 1995; Jones 1996). The extent to which plants infected by contact can act as secondary foci for spread within pastures is influenced similarly by grazing pressure. When this is heavy, they remain accessible to stock rather than becoming overshadowed by neighbouring healthy plants. Clover plant density also influences rate of spread, as, when it is low, fewer infections are needed to establish high incidences. Other factors favouring increased spread and high final incidences in infected pastures include warmer than normal temperatures during winter that favour greater virus concentration within infected plants making them more potent sources for spread, high incidences of clover versus non-host species that retard the epidemic in mixed species swards, intensive grazing of restricted areas and growing seasons extended by late rains allowing spread to continue (Ferris and Jones, 1994, 1995; Jones, 1996; McKirdy et al., 1998; Jones et al., 2001; Jones 2004a).

BYMV: BYMV occurs worldwide, infects many crop, pasture, weed and wild legume species and many non-legumes including both monocots and dicots, and is transmitted non-persistently by many aphid species regardless of whether they colonise legumes or not (e.g. Boswell and Gibbs 1983). Johnstone and McLean (1987) reported that known BYMV vectors that colonise legumes in Australia include the pea aphid (*Acyrthosiphon pisum*), thew cowpea aphid (*Aphis craccivora*), the cotton aphid (*Aphis gossypii*), the foxglove aphid (*Aulacorthum solani*) and potato aphid (*Macrosiphum euphorbiae*). In mixed species, subterranean clover-based pastures in south-west Australia, bluegreen aphid (*Acyrthosiphon kondoi*), cowpea aphid (*Aphis craccivora*) and spotted clover aphid (*Therioaphis trifolii*) colonise clovers and other legume species, green peach aphid (*Myzus persicae*) mainly colonises broad-leafed weeds and the oat aphid (*Rhopalosiphum padi*) and maize or corn leaf aphid (*R. maidis*) colonise grasses (Ridsdill-Smith and Scott 1997; Thackray and Jones 1999). Turnip aphid (*Lipaphis erysime*) flying from wild radish (*Raphanus raphanistrum*) weeds or oilseed rape (*Brassica napus*) crops often probes pasture plants whilst migrating. When moving between plants within pastures, winged forms of the three clover- colonising species and of green peach aphid, oat aphid, maize aphid, and turnip aphid have the opportunity to probe BYMV-infected clovers and acquire the virus before flying off, probing and infecting other clovers, the non-colonising species then moving on in search of their preferred hosts. In addition, regardless of whether or not they colonise clover, non-winged aphids moving between adjacent, intermingling clover plants within swards acquire and transmit the virus locally. All these aphid species can transmit BYMV from clover at differing transmission efficiencies. Thus all of them are probably involved to differing extents in transmission of BYMV from clover to clover within subterranean clover- based pasture, the actual vector species transmission scenario varying with site and year (McKirdy and Jones 1995a; Ferris and Jones 1996; Jones and Ferris 1999, 2000; Jones 2004a).

Seed transmission of BYMV is recorded at low levels in subterranean clover (R.A.C. Jones, unpubl.), other annual clovers and a few naturalised wild annual clover species, with 1% the maximum rate of transmission to seedlings found. The virus ‘over summers’ in annual pastures inside dormant seeds within the clover seed bank (McKirdy and Jones 1995; McKirdy et al. 2000). When infected clover seeds germinate they produce seed-infected plants from which aphids acquire the virus and spread it to healthy plants. Subsequent spread by aphid transmission is polycyclic. If virus spread starts early, almost all clover plants may become infected by the end of the growing period (Jones 1992, 1994, 1996). Grouping of infected plants around primary infection foci resulting in patches of infection that expand is characteristic of diseases that are transmitted non-persistently by aphids. This pattern of spread occurs around BYMV-infected plant foci in grazed subterranean clover swards, but spread also develops further away resulting in new infection foci consisting of scattered affected plants or such plants becoming centres of new patches. When final BYMV incidence approaches 100%, the infected patches coalesce. Wingless aphids walking between intermingling pasture plants expand the patches but spread by winged aphids initiates new infection foci. Although the virus is endemic in much of south-west Australia, spread to new sites is still possible through sowing infected seed stocks of subterranean and other annual clovers (Jones 1994, 1996, 2004a; McKirdy and Jones 1995a; McKirdy et al. 2000).

Similar factors to those described for SCMoV influence the magnitude of the BYMV source in pastures due to seed-borne infection and its accessibility to aphid vectors (see above). When spread of BYMV starts early due to early arrival of aphids and their rapid colonisation of swards, there are many seed- infected clover plants and heavy grazing prevents shading out of virus-infected clovers, a rapid take off of the exponential phase of virus spread occurs, final incidence is high and overall yield losses are greatest (Jones 1992, 1994, 1996, 2004a; Ferris and Jones 1994, 1995; Thackray and Jones 1999). The earlier spread starts the greater the final BYMV incidence reached before plants die from insufficient moisture. Rainfall is critical in determining when aphids first arrive and initiate virus spread. Higher than normal late summer and early autumn rainfall is associated with years of greater virus spread and virus-induced yield losses because such rains cause early germination and growth of pasture plants. This allows aphids to multiply under the warm conditions. In contrast, a dry start to the growing season means that pastures germinate late after the first substantial rains, which are delayed until conditions are too cold for rapid aphid build-up and virus spread. Hence the BYMV epidemic starts late and relatively few plants are infected by the end of the growing period. Other factors favouring BYMV epidemics include low plant density, warm and wet conditions in winter and extended growing seasons. Thin swards with bare earth exposed between plants attract greater numbers of colonising and non-colonising aphids to land than swards where the ground is covered over. Also, as with SCMoV, with low plant density fewer infections are needed to establish high incidences. Cool conditions in winter limit aphid multiplication and activity. They also decrease virus concentration in infected source plants and therefore its acquisition by aphids. As with SCMoV, dry conditions during the growing period result in greater mortality of infected than healthy plants while growing seasons extended by late rains prolong the life of swards allowing spread to continue for longer (Jones 1994, 2004a).

CMV: CMV was first isolated from subterranean clover cv. Enfield plants in WA in 1982, and subsequently in subterranean clover plots in VIC and TAS (Garrett 1985; Johnstone and McLean 1987). CMV is transmitted non-persistently by many aphid species regardless of whether they colonise legumes. Blue-green aphid (*Acyrthosihon kondoi*) is the commonest and most important aphid vector within subterranean clover plots and grazed subterranean clover pasture swards (Jones and McKirdy 1990; Jones 1991). Green peach aphid *(Myzus persicae*) is also an important vector in subterranean clover breeding and experimental plots (McKirdy and Jones 1994b). CMV is readily seed-borne in subterranean clover, seed transmission occurring at different rates in different varieties. Seed samples collected from CMV-inoculated plants of 11 different subterranean clover varieties transmitted CMV through seed at different rates. The highest rate was 9% but seed transmission rates >5% were only obtained with cvs Enfield, Green Range and Nangeela. Transmission rates <1% were found in cvs Esperance, Junee, Seaton Park., Tallarook and Woogenellup. Seed transmission rates reflected the susceptibility differences found between varieties (Jones and McKirdy 1990)

The only source of CMV of any consequence for annually self-regenerating subterranean clover pastures in agricultural regions of south-west Australia is the sown clover seed itself (Jones and McKirdy 1990; Jones 1991, 2000; McKirdy and Jones 1994b). Jones (1991) described the dynamics of the CMV epidemics that developed when aphids spread CMV from initial seed-borne sources to healthy plants within grazed subterranean clover swards. They resemble those described for BYMV so will not be described in detail here. In brief, sowing virus-infected clover seed introduces sources of virus within the growing sward through presence of seed-infected plants. Aphid vectors acquire the virus from these primary foci and spread it to healthy plants within the sward. The pattern of CMV spread found was consistent with spread by wingless colonising aphids walking from plant to plant with movement over distance by winged aphids resulting in initiation of new infection foci occurring only rarely. The extent of CMV spread depended on the level of infection in the seed sown, availability of soil moisture for establishment of the weaker CMV-infected seedlings, and the time of arrival, abundance and activity of blue-green aphid (*Acyrthosihon kondoi*) in the swards. Also, increasing prevalence of grass weeds increasingly slowed virus spread in the clover component of self-regenerated swards. Moreover, annual fluctuations in CMV detection near the end of the growing season were also influenced by other key factors such as length of growing season (Jones 1991, 2000).

SCSV: SCSV was first found in Australia in 1950 (Grylls and Peak 1960). It occurs in TAS, NSW and VIC. Although Buchen-Osmond et al. (1988), Harvey (1958) and Shipton (1967) reported this virus causing a disease in pastures in south coastal areas of WA, it is now clear that these reports were due to confusion with stunting by SCMoV and presence of SCSV in WA has not been confirmed. Different isolates differ in the severity of the symptoms they induce and their ability to infect different subterranean clover varieties (O'Loughlin Grylls and Peak 1960, 1969; Johnstone 1983), host ranges and vector specificities (Grylls and Butler 1958; Smith 1966; Johnstone and Patten 1981; Johnstone and McLean
1987). SCSV is transmitted persistently by the cowpea aphid (*Aphis craccivora*). Winged cowpea aphids cause sporadic outbreaks of SCSV as they migrate to and within south east Australia and TAS (Grylls 1972; Smith 1966; Wade 1957). Alternative hosts of SCSV include burr medic, small woolly burr medic (*M. minima*), black medic (*M. lupulina*), spotted medic (*M. arabica*), drooping flowered clover (*T. cernuum*), cluster clover (*T. glomeratum*), suckling clover (*T. dubium*) (Buchen-Osmond 1988).
Epidemics depend on migration of its cowpea aphid vector and severe outbreaks occur sporadically (Johnstone and McLean 1987). SCSV epidemiology was studied extensively and a model developed to explain the dynamics of the pathosystem (Gutierrez et al. 1971, 1974a,b). Weather is a critical determinant in south-east Australia as the cowpea aphid vector cannot tolerate cold conditions and has a high temperature threshold for development of 8^{o}C. It overwinters on small woolly burr medic (*M. minima*) and burr medic (*Medicago polymorpha*) plants in south-east QLD and northern NSW (Johnstone 1957; Gutierrez et al. 1974b). The cowpea aphid vector contracts to cooler wetter areas of south-east Australia during summer and spreads again during autumn before contacting northwards with the onset of winter (Johnstone and McLean 1987). The cowpea aphid can migrate more than 1000 Km (Johnstone 1984a). It has a very high rate of population increase on favourable hosts and this permits it to exploit brief periods of favourable conditions in different regions (Johnstone and McLean 1987).

SCRLV: SCRLV was first reported infecting subterranean clover in VIC in 1965 (Anon. 1968). It is now considered to be a strain of *Soybean dwarf virus* (Johnstone et al. 1982; Ashby and Johnstone 1985; Johnstone and MacLean 1987). SCRLV is not seed-borne, but survives the summer in infected perennial white clover, red clover and lucerne pastures. SCRLV also infects many other legume species including many pasture legumes (Kellock 1971; Johnstone et al. 1944a). It is transmitted persistently by the pea aphid (*Acyrthosihon pisum*), the foxglove aphid (*Aulacorthum solani*) and the potato aphid (*Macrosiphum euphorbiae*), but the foxglove aphid is the most important vector in clover pastures (Kellock 1971; Johnstone 1978; Johnstone and Patten 1981; Helms et al. 1983). Reservoirs of SCRLV infection are also found in certain species of weeds, especially storksbills (*Erodium* spp.) and docks (*Rumex* spp.) and these can act as sources for spread by aphids to subterranean clover pastures (Johnstone et al. 1884a; Johnstone and Duffus 1984), In TAS, development of SCRLV epidemics in spring and in autumn sown plantings is often mostly due to spread by wingless aphids but high infection levels soon after the autumn break to the season results from inoculations by winged aphids (Johnstone and Rapely 1979, 1981).

9.4.5 Current Management Strategies – viral diseases

SCMoV: As mentioned above, there is natural genetic resistance to SCMoV in some commercial varieties of subterranean clover. This resistance is based on restricted cell-to-cell movement of the virus (Wroth and Jones 1992a; Njeru et al. 1995; Fosu-Nyarko et al. 2002). When the effectiveness of resistance previously identified in different varieties of subterranean clover by inoculation with infective sap in the glasshouse was tested in field experiments with grazing sheep, strong resistance was not overcome and partial resistance greatly diminished spread of SCMoV in some varieties. However, in other partially resistant varieties the epidemic rate approached that found in susceptible varieties indicating resistance breakdown (Ferris et al. 1996). Similar results were obtained in simulated trampling and grazing experiments in the glasshouse with varieties differing in resistance type (McKirdy et al. 1998).

Although field experiments that measured yield losses when virus was spread by grazing sheep demonstrated the impacts of altering grazing pressure on SCMoV incidence in subterranean clover swards (Ferris and Jones 1995), the cost of pasture experiments with grazing meant that information from field experiments on the effectiveness of individual control measures was available only for resistant varieties (Ferris et al. 1996). Therefore, construction of an appropriate mix of control measures to include in Integrated Disease Management (IDM) approaches for SCMoV in grazed subterranean clover pasture was based mainly on epidemiological information. Only phytosanitary, cultural and host resistance control measures are available. As a vector is not involved, no chemical or biological measures are possible.

A key control measure included in Integrated Disease Management (IDM) is sowing SCMoV-resistant clover varieties in pastures. Clover varieties that are highly SCMoV-resistant resistant are recommended as, s mentioned above, partial resistance may be ineffective under heavy stocking pressure (Wroth and Jones 1992a; Njeru et al. 1995; Ferris et al. 1996). Other control measures include: avoiding new virus introductions by sowing new pastures with tested clover seed stocks that have no contamination with SCMoV; avoiding introduction of SCMoV to healthy pastures on farm machinery or stock moving from older infected pastures; sowing pastures with admixtures of susceptible clover varieties with resistant ones or with alternative pasture species that are non-hosts (e.g. grasses); relaxing grazing pressure to (i) minimise contact spread by stock and (ii) allowing swards to grow up in spring so that healthy plants shade out the stunted SCMoV-infected source plants; avoiding driving vehicles over pastures to prevent SCMoV spread on wheels; decreasing the seed bank of SCMoV-infected clover seeds in the soil by growing crops instead of pastures in affected fields for several years in extended (phased) rotations; and avoiding sowing new pastures in fields next to old infected ones (Jones 2004a).

BYMV: As mentioned above, varieties of subterranean clover differ in susceptibility to BYMV. Karridale, Green Range, Trikkala, Meteora and Mt Barker are among the more susceptible, but Esperance is slower to become infected. There is little natural resistance to BYMV among commercial varieties of subterranean clover, with strain-specific, systemic hypersensitive resistance to the virus only confirmed in only one outmoded variety, cv. Rosedale (Ferris and Jones 1996).

Research overseas identified sources of resistance to BYMV in Alsike and red clover. BYMV resistant red clover varieties have been bred in the USA. Studies on the inheritance of BYMV resistance in red clover were also published (reviewed by Taylor and Ghabrial 1986).

The critical epidemiological information described above was used to devise an Integrated Virus Disease Management (IDM) strategy to control BYMV in grazed subterranean clover-based pastures (Jones 2004a). In general, the control measures closely resemble those recommended for SCMoV, except where an individual measure targets the method of virus transmission (contact or aphid). As with SCMoV, because of the complexities and expense of pasture experiments with grazing animals, the component measures are based mainly on a detailed understanding of the factors that drive epidemics.

However, specifically designed field experiments were used to assess rates of BYMV spread in different subterranean clover varieties (Ferris and Jones 1996). Also, the recommendation to spray pyrethroid insecticide to kill vector aphids in late autumn/early spring comes by extrapolation from field experiments in which they suppressed spread of AMV in grazed annual burr medic (*Medicago polymorpha*) swards (Jones and Ferris 2000). Because of the greater role played by wingless aphids as virus vectors in pasture than in annual crops, insecticide controls non-persistently aphid-borne viruses more effectively in grazed pasture than in crops. Moreover, it is normal to spray to kill pasture pests in spring, so its use to control BYMV is unlikely to raise additional environmental concerns (Jones and Ferris 2000). Field experiments designed to measure yield losses demonstrated shading out of BYMV-infected clover source plants through competition with healthy clover plants in undefoliated swards (Jones, 1994). The control measures in the Integrated Disease Management (IDM) package include: avoiding new virus introductions by sowing new pastures with tested clover seed stocks that have no contamination with BYMV and avoiding sowing new pastures next to old infected pastures; sowing more clover into sparse pastures to increase plant density and reduce aphid landing rates; sowing early maturing or less BYMV susceptible varieties; sowing pastures with admixtures of susceptible clover varieties with pasture species that are non-BYMV hosts (e.g. grasses); relaxing grazing pressure to allow swards to grow up in spring so that healthy plants shade out the stunted BYMV-infected source plants; decreasing the seed bank of BYMV-infected clover seeds in the soil by growing crops instead of pastures in affected fields for several years in extended (phased) rotations; and spray pasture with pyrethroid insecticide to kill aphid vectors in late winter/early spring (Jones 2004a).

<u>*CMV*</u>: Field studies in south-west Australia determined ‘threshold’ levels for seed-borne CMV in subterranean clover (Jones 1988, 1991, 2000; McKirdy and Jones 1994b) Rather than sowing new experiments each year as with annual crops, this work involved sowing seed at two sites and following the seed-borne virus infection scenarios that unfolded in these same plots every year over a period of several years. A ‘threshold’ CMV seed infection level for sowing of <0.5% was established (Jones 1988, 2000). Even in more aphid-prone high rainfall areas, this was considered adequate to avoid the risk in later years of economic losses due to any decrease in subterranean clover feed for livestock resulting from CMV infection. A testing service for CMV in samples of subterranean clover seed is available. It is used to test seed before release of seed of new varieties, and involves testing of 1000 seeds/sample for CMV seed transmission (Jones 2000). Sowing seed of less CMV-susceptible subterranean clover varieties is also possible but other control measures have not been considered as yet.

<u>*SCSV*</u>: Cv. Tallarook and some subterranean clover accessions were highly resistant or immune to SCSV (O’Loughlin 1958; Grylls and Peak 1960), and cv. Howard was bred specifically for SCSV resistance (Anon. 1997). Unfortunately, SCSV is highly variable (Grylls and Peak 1960; Stovold 1983a; Johnstone and McLean 1987) and cvs Howard or Tallarook were both susceptible to SCSV isolates obtained later from southern Australia (Johnstone

1983). Additional sources of SCSV resistance were the identified and used in breeding for resistance (Gladstones and Collins 1983; Johnstone 1983; Johnstone and McLean 1987).

SCRLV: Seed lines of subterranean clover with tolerance to SCRLV were used in resistance breeding in Australia (Johnstone and Barbetti 1988). Insecticides can be used to prevent winged aphids making new infections with SCRLV in pastures (Johnstone 1995, 1984b). Avoiding sowing new subterranean clovers next to old white clover, red clover or lucerne pastures is advised as the latter provide sources of SCRLV for spread to subterranean clover (Johnstone and McLean 1986).

9.4.6 What are the gaps and research needed?

The critical gaps that need addressing in relation to foliar diseases of annual clovers for southern Australia are as follows:

Define the race structure for the clover scorch pathogen *Kabatiella caulivora* across Australia, and associated host resistances against individual and combined races of *K. caulivora* such that available host resistances can be strategically deployed

Define the strains of SCMoV, BYMV and AMV occurring in annual pasture clovers across Australia such that available host resistances can be effectively deployed

Survey all non-subterranean clover annual pastures to establish relevant benchmarks for foliar fungal disease and viral incidence and define yield impacts

Survey subterranean clover pastures for SCMoV, BYMV, SCSV, SCRLV to establish relevant benchmarks for viral incidence and obtain yield loss data from grazed swards for all important viral pathogens in annual clover pasture species, except for SCMoV, CMV and BYMV in subterranean clover and AMV in annual medics

For major diseases, define relative resistances of all near-release breeding genotypes across all annual clover pasture species: e.g. for annual clovers to Kabatiella, Cercospora, rust, powdery mildew, SCMoV and BYMV and where required, identify useful sources of resistances to these pathogens for breeders to utilise

Ensure seed stocks of new variety releases of annual clover pasture legumes are free of seed- borne virus infection

9.5 Perennial clovers

9.5.1 Soil-borne diseases of perennial clovers

9.5.2 Introduction

Fungal and oomycete soil-borne diseases of perennial clovers, such as white clover, have not previously been reviewed in Australia.

9.5.3 Disease symptoms, scope, extent and impact of soil-borne fungal, oomycete and nematode diseases on pasture productivity and pathogens involved

Fungal and oomycete disorders: Fungal and oomycete disease symptoms on roots of white (*Trifolium repens*) and other perennial clovers generally approximate those widely seen on annual clovers and many of the same soil-borne pathogens also attack perennial clovers (Clarke 1999d). However, in comparison with annual clovers, relative little information is available, with root rot disorders generally accepted to be of common occurrence despite the relatively little research investigation have been undertaken. Studies both in Western Australia (Maughan and Barbetti 1983) and eastern Australia (Willams and Pascoe 1994; McMullen et al. 1997) indicated the importance of various soil-borne fungi and oomycetes, including species of *Rhizoctonia, Fusarium, Phytophthora* and *Pythium* in the decline ofwhite clover from pastures. In particular, *Phytophthora megasperma* was shown to be a serious root pathogen of white clover in the northern irrigation areas in Victoria (Williams and Pasco 1994).

These earlier studies in total suggest that in temperate areas of Australia, *P. megasperma*, *Pythium* spp. and *Rhizoctonia* spp. are the likely most important causal agents of root rot of white clover (Irwin and Jones 1977; Maughan and Barbetti 1983; Williams and Pascoe 1994).

Fungal disease in particular on white clover can often be associated with damage to both roots and to stolons and can lead to the subsequent death of these plant structures, as occurred in Western Australia (Maughan and Barbetti 1983) where such combined root and stolon rot caused by *Rhizoctonia* was important in the irrigation areas. Similarly, in Queensland Irwin and Jones (1977) reported a combined root and stolon rot of white clover that involved brown discolouration of tap and lateral roots extending into the vascular tissue and often resulting in the total rotting of affected roots and associated stolons. In their study, they also consistently isolated *Pythium middletonii* from necrotic stolons and roots of *Trifolium* spp. More recently, Zahid et al. (2001a) undertook a survey of 12 white clover-based dairy pastures on the north coast of NSW and south-eastern Queensland and detected species of *Fusarium, Codinaea, Colletotrichum, Drechslera, Rhizoctonia, Phoma, Pythium,* and *Phytophthora* from roots and/or stolons of white clover. They found that fungal rots of roots and stolons were most severe during the summer months. They concluded that these pathogens are likely to be contributing towards the poor seedling performance, growth and persistence of white clover typical in pastures of the subtropical east coast of Australia and this was further supported by subsequent pathogenicity studies with the fungal and oomycete pathogens (Zahid et al. (2001b), with several species of fungi shown to be pathogenic to white clover roots and stolons (Zahid et al. 2001b).

Nematode disorders: Nematodes adversely affect white clover growth (Colman 1964; Zahid et al. 2001c) and persistence of white clover. This is evidenced by the fact that nematicide application enhances the yield of white clover in Australian (Irwin and Jones 1977; McMullen et al. 1997). Widespread occurrence of a root-knot nematode *Meloidogyne trifoliophila* (Zahid et al. 2000) in the subtropical east coast of Australia (Zahid et al. 2001a) and its possible involvement in disease complexes (Irwin and Jones 1977; McLeish et al. 1997) emphasises the importance of management of this nematode in pastures. More recently, when Zahid et al. (2001a) undertook a survey of 12 white clover-based dairy pastures on the north coast of NSW and south-eastern Queensland, they detected six nematode species. They found that root-knot symptoms caused by plant parasitic nematodes were more severe in June.

Sedentary endoparasitic nematodes such as *Meloidogyne trifoliophila*, *Heterodera trifolii* and the ectoparasitic nematode *Helicotylenchus dihystera* were the numerically dominant nematodes in that region. Other nematode species, including *Pratylenchus, Xiphinema* and *Tylenchorhynchus*, were also present but at lower frequencies and they used principal component analysis to show that the latter were less important as white clover pathogens. *M. trifoliophila* was a first record of this species in Australia and was found to be present at all sites. They concluded that these nematodes were, in addition to root and stolon rots, also likely contributing significantly to the poor seedling performance, growth and persistence of white clover typical in pastures of the subtropical east coast of Australia.

9.5.4 Current Management Strategies – Fungal, oomycete and nematode disorders

Although cover crops showed potential for improving yield and in reducing the population of certain nematodes, removal of white clover pasture for 5 months as a management practice does not appear to be useful in overcoming root and stolon rot diseases of white clover in this subtropical climate (Zahid et al. 2002).

9.5.5 What are the gaps and research needed

The critical gaps that need addressing in relation to soil-borne diseases of perennial clovers for southern
Australia are as follows:

Survey perennial clover pastures across southern Australia to establish relevant benchmarks for soil-borne disease incidence and impacts.

Define relative resistances of current varieties and near-release breeding genotypes across all perennial clovers to the most important of the soil-borne root pathogens.

Identify useful sources of resistance to the most important of the soil-borne root pathogens for breeders to utilise.

9.6 Foliar diseases of perennial clovers

9.6.1 Introduction

Virus diseases of white clover pastures in Australia were reviewed most recently by Johnstone and Chu (1993) and Jones (1996), but research undertaken subsequently on virus diseases of white clover has not been reviewed. There has been little Australian research on virus diseases of other perennial clovers. Fungal and oomycete foliar diseases of perennial clovers have not previously been reviewed in Australia and relatively little research has been undertaken in comparison with the large amount on subterranean clover.

9.6.2 Disease symptoms, scope, extent and impact of fungal foliar diseases on pasture productivity and fungal pathogens involved

The vast majority of fungal genera pathogenic on annual clovers are, to some extent, also pathogens on perennial clovers, particularly white clover (Clarke 1999d). Some of the more commonly occurring and damaging foliar pathogens include the following:

Rust: Typical rust (caused by *U. trifolii-repentis* and/or *U. nerviphilus*) pustules formed on undersides of leaves and sometime on stems and petioles. Rust on white clover in New South Wales, can cause serious defoliation during late winter and early spring, especially in coastal districts (Anon. 1966).

Black blotch: Black blotch or sooty blotch, caused by the fungus *Cymadothea trifolii*, results in black sooty lesions on the leaves Black blotch is a common disease of white clover in Victoria (Clarke 1983b, 1999e) but rare in NSW (Valder 1954) where it also affects red clover (Butler 1953). It causes dark brown or black angular blotches, mainly on the under surface of the leaves. Infected leaves remain green at first, then dry, turn brown and finally die. Although it rarely causes serious herbage loss (Butler 1953; Clarke 1983b) in some situations it can affect pasture yields by stunting and partly defoliating the affected plants (Butler 1953; Barbetti 1983d). Unless heavy infection occurs, when the stand should be cut and the affected hay removed, there generally is no necessity to adopt any control measures (Butler 1953).

Sclerotinia: The fungus *Sclerotinia trifoliorum* causes clover rot and can have a great impact on white clover productivity. Outbreaks occur most years although the incidence and severity of the disease varies from year to year.*Other minor diseases*: Other minor fungal diseases include grey mould, caused by the fungus *Botrytis cinerea*, and wart disease, caused by the fungus *Physoderma trifolii* (Clarke 1999d). Fungi that cause leaf spot diseases in white clover include *Leptosphaerulina trifolii* (Pepper spot), *Pseudopeziza trifolii* (Common leaf spot), *Stemphylium* spp. (Stemphylium leaf spot), *Stagonospora* spp. (Stagonospora leaf spot), *Peronospora trifoliorum* (Downy mildew) and *Erysiphe trifolii* (Powdery mildew), but all rarely cause significant losses (Clarke 1999e).

9.6.3 Current Management Strategies – Fungal foliar diseases

Other than use of fungicides in cost-effective situations and use of heavy grazing to make the sward less conducive for severe foliar disease (e.g., rusts, powdery mildew) little is available by way of effective management options for control of foliar fungal pathogens on perennial clovers. Some varieties and different species are known to be relatively more or less susceptible to some particular pathogens.

9.6.4 Disease symptoms, scope, extent and impact of virus diseases on pasture productivity and viral pathogens involved

The textbook ‘Viruses of Diseases of Plants in Australia’ by Buchen-Osmond et al. (1988) lists all viral pathogens then known to infect perennial pasture clover species in Australia. Virus diseases of white clover pastures in Australia were reviewed by Johnstone and Chu (1993). White clover viruses were also covered briefly in general pasture virus reviews by Johnstone

and Barbetti (1987), Sward (1990), Panetta et al. (1993) and Jones (1996). Surveys of white clover pastures in Tasmania, eastern Australia and south-western Australia provided detailed information on their incidence, and which are important (Johnstone and Duffus 1984; Garrett 1991; McKirdy and Jones 1995b, 1997; Norton and Johnstone 1998; Coutts and Jones 2002). Apart from studies on effects on nodulation (Guy et al. 1980) and brief mention by Garrett (1991), no studies on losses due to virus infection in white clover are available from
Australia, but such studies have been undertaken overseas (Gibson et al. 1982; Campbell 1984; McLaughlin et al., 1992; Potter 1993; Brink and McLaughlin 2002; Brink et al. 1996). Godfree et al. (2004) reported on the incidence of viruses in naturalised sub-alpine populations of white clover in south- east Australia. Perennial clovers other than white clover have been relatively little studied but some limited information is available for red and strawberry clovers (Buchen-Osmond et al. 1988; McKirdy and Jones 1995a; Jones 1996).

9.6.5 Disease symptoms – viral pathogens

The viruses infecting perennial clovers in pastures in Australia are:-

White clover

Most important viruses

Alfalfa mosaic virus (AMV): AMV infection causes marked symptoms of interveinal light green or yellow mottle on leaves and stunting of the infected plant. Symptoms vary from light, and almost unnoticeable, to severe, causing plants to die, due to the diversity of strains of the virus, the variety and environmental conditions. Symptoms may be masked by hot weather.

White clover mosaic virus (WCMV): WCMV causes marked leaflet symptoms of vein clearing, vein yellowing, small yellow ringspots and diffuse yellow mosaic symptoms. However, these symptomsseldom occur on all leaves and are more noticeable on actively growing plants in the spring and autumn. They can be masked by cold weather or in plants lacking vigour. Affected plants lack vigour but rarely die.

Common viruses that cause few or no symptoms are as follows:

Clover yellow vein virus (CYVV): Infected plants are generally without symptoms, but young infected leaves show transient vein clearing or vein yellowing during spring and autumn.

Subterranean clover red leaf virus (SCRLV): SCRLV causes symptomless infection of white clover plants.

Minor viruses are as follows:

Beet western yellows virus (BWYV); *Cucumber mosaic virus* (CMV); *Lucerne Australian latent virus* (LALV); *Pea pimple pod virus* (PPPV); *Red clover necrotic mosaic virus* (RCNMV); *Rugose leaf curl virus* (RLCV); *Subterranean* clover stunt virus (SCSV)

Red clover

The most important virus disorders of red clover are as follows:

Bean yellow mosaic virus (BYMV): Infected plants show symptoms of leaf mottle, leaf deformation and plant stunting.

<u>Minor viruses are as follows: </u>*Alfalfa mosaic virus* (AMV); *Cucumber mosaic virus* (CMV); *Red clover necrotic mosaic virus* (RCNMV); *Subterranean clover red leaf virus* (SCRLV), *Subterranean clover stunt virus* (SCSV)

Strawberry clover

The only record of virus disorders occurring on strawberry clover is White Clover Mosaic Virus (WCMV). Only the commoner viruses of white and red clover will be discussed in more detail below.

9.6.6 Extent and impact of virus diseases on productivity

Incidences of viral infection in older stands of white clover are often high (Clarke 1999c; McKirdy and Jones 1995b, 1997; Norton and Johnstone 1998; Coutts and Jones 2002). In TAS, surveys revealed average infection incidences with SCRLV of 37% in white clover pastures (Johnstone and Duffus 1984). In eastern Australia, AMV and WCMV were the most common and widespread viruses (Norton and Johnstone 1998). They infected plants at many sites soon after establishment and then rapidly increased to high levels, occasionally exceeding 99% of plants. In 1994, moderate levels of SCRLV infection were found at most southern, winter-rainfall dominant sites surveyed. The occurrence of CYVV was sporadic and infection levels were always low (Norton and Johnstone 1998). In south-west Australia, when the incidences of AMV and SCRLV were surveyed in white clover, AMV was detected in most pastures with many having infection levels >86%, while SCRLV was found in several at infection levels of <12% (McKirdy and Jones 1995b). However, none of the pastures with high levels of AMV infection had been re-sown with white clover within the last 20 years, whereas those re-sown within the last five years had little or no infection (McKirdy and Jones 1995b). In a subsequent survey, McKirdy and Jones (1997) again found widespread infection with AMV and occasional infection with SCRLV. They also found thatinfection with WCMV was widespread with incidences up to 83% and most pastures were also infected with CYVV at infection levels of up to 23%. None of the white clover pastures with a high incidence of WCMV had been re-sown with white clover within the last 10 years, whereas those re-sown within the last 5 years had little or no infection. Many white clover plants within pastures had 2 or more viruses, a few having all 4 viruses (McKirdy and Jones 1997). By contrast, when virus incidences in naturalised sub-alpine populations of white clover were examined in south-east Australia, CYVV was by far the commonest virus with incidences up to 59%, with AMV and WCMV only occasionally found (Godfree et al. 2004).

Red clover is severely affected by infection with BYMV in pastures, but white and strawberry clovers do not become infected with this virus (McKirdy and Jones 1995a; Jones 1996). No surveys of virus incidence in red or strawberry clover have been undertaken in Australia.

Studies undertaken overseas demonstrated considerable losses in white clover herbage and seed production, nitrogen fixation, pasture persistence and the ratio of desirable to undesirable pasture species caused by virus infection (e.g. Gibson et al. 1982; Campbell 1984; Barnett and Diachun 1986; McLaughlin et al., 1992; Potter 1993; Brink and McLaughlin 2002; Brink et al. 1996). In Australia, glasshouse studies with white clover found dry weight losses of 24% due to the presence of WCMV (Garrett 1991), and WCMV infection reduced root nodule numbers by 71% (Guy et al. 1980). No studies have been done to quantify the effects of AMV on white clover in Australia. These reports need to be followed up with more in depth studies to clarify the effects of infection with AMV and WCMV on productivity of grazed white clover pasture swards in Australia.

In 1991, it was estimated that the agronomic impact of viruses on white clover equates to a loss of $30 million annually to just the Australian diary industry (Garrett 1991), but losses to the meat industry were not estimated. Based on overseas loss data, Kalla et al. (2001) suggested that in the field in Australia AMV, CYVV and WCMV may reduce Australian white clover pasture production by up to 30% through reduced foliage yield and quality, reduced nitrogen fixing capacity and reduced vegetative persistence.

9.6.7 Aetiology and epidemiology of viral pathogens

AMV and CYVV are transmitted non-persistently by aphids. Spread of AMV in white clover pastures increased considerably following the introduction to Australia in the late 1970s of seed lots of lucerne carrying AMV, and of three species of pasture aphids, the blue-green, spotted alfalfa and pea aphids, all of which transmit AMV (Garran and Gibbs 1982; Hajimorad and Francki 1988; Jones 1966). SCRLV is transmitted persistently by the foxglove and pea aphids (Johnstone and McLean 1987; Johnstone and Chu 1993). AMV spread within a stand is dependent on aphid vector numbers. In rainfed pastures in south-east Australia, its spread is enhanced in years with summer and autumn rains which favour plant growth and thus an increase in aphid numbers, and dry springs which favour aphid activity and the development of symptoms. Aphid vector numbers are kept down in years with cold wet weather or the presence of aphid parasites (Clarke 1999c).

WCMV is not aphid-borne but is readily spread by contact. McKirdy et al. (1998) examined the effectiveness of mowing, and of grazing and trampling by livestock, at spreading WCMV in white clover swards in WA. Grazing and mowing were more effective at spreading WCMV than trampling. WCMV is transmitted by contact during grazing and trampling by livestock, tractors and mowers. No vectors are involved.

Modern pasture management systems optimise use of available feed by employing short duration, rotational heavy grazing of restricted areas to minimise wastage of feed and by mowing pastures to prevent them becoming ‘rank’ and less appetising for stock (McKirdy et al. 1998; McKirdy and Jones 1999). Such systems favour spread of contact-transmitted viruses like WCMV. They also favour spread of vector-borne viruses like AMV by preventing shading over by vigorous healthy plants of the weaker virus-infected plants that act as sources for virus acquisition by vectors (Coutts and Jones 2002).

When Coutts and Jones (2002) tested samples from 6 mixed grass and white cover pastures in WA at regular intervals over periods of 10-22 months, AMV incidences ranged from 0% to 100% while WCMV incidences fluctuated between 9% and 46%. During repeated trapping of aphid vectors of AMV, numbers of aphids caught varied widely between trapping dates and individual pastures. The species caught were blue green-aphid (*Acyrthosiphon kondoi*), pea aphid (*A. pisum*), cowpea aphid (*Aphis craccivora*), oat aphid (*Rhopalosiphum padi*) and spotted alfalfa aphid (*Therioaphis trifolii*).

When viruses infect their respective hosts within a mixed population of plant species, as in commercial white clover pastures, a dynamic balance exists for each virus between increasing incidence due to infection of further healthy host plants and decreasing incidence resulting from poor competitive ability and survival of infected plants. There were often wide fluctuations in incidence of AMV and WCMV in the white clover component of such pastures (Coutts and Jones 2002). Factors that favour spread of infection to healthy host plants by contact (WCMV) or aphid vectors (AMV) increase virus incidence. These include ones that favour growth and survival of infected plants or virus multiplication within them thereby ensuring that a substantial virus source is present, and ones that favour increased vector abundance or plant wounding by stock and mowing. Declining virus incidence results from factors that (i) increase the invasiveness and competitive ability of non-host plant species, or of healthy plants of host species, at the expense of growth and survival of infected plants, (ii) diminish virus concentration within infected plants, or (iii) decrease vector numbers or plant wounding by stock or mowing. Of these, (i) and (ii) are important because when the proportion of infected plants or virus concentration within them decreases, the source of virus inoculum for further spread also declines, and (iii) because less virus transmission occurs to healthy host plants. Virus incidence declines when new infections fail to keep up with loss of infected plants (Coutts and Jones 2002).

9.6.8 Current management Strategies - viral diseases

There are no reports of natural resistance genes to AMV or WCMV being sought in white clover varieties or breeding lines in Australia, but resistance to AMV and CYVV was found in white clover in the USA and used in breeding for virus resistance (Barnett and Gibson 1975; Gibson and Barnett 1977; Taylor et al. 1977; Taylor and Ghabrial 1986). However, transgenic resistance to AMV and CYVV was developed in white clover in Australia (Kalla et al. 2001). A study to establish the risks of releasing this transgenic material was made subsequently which indicated that escape of the transgenic virus-resistant white clover might be detrimental to native plants because they might compete more effectively against native plants than non-transgenic naturalised white clover (Godfree et al. 2004).

Varieties of red clover with resistance to BYMV have been bred overseas and other red clovers not specifically bred for virus resistance have been found to be BYMV-resistant (Taylor and Ghabrial 1986). Virus resistance in red clover has not been studied in Australia.

Pyrethroid insecticides are effective at suppressing spread of non-persistently aphid–borne viruses like AMV in pasture swards but not crops (Jones and Ferris 2000). This is because most spread is by wingless forms of aphids within swards but winged often non-colonising aphids in crops. Increasing the proportion of non-virus hosts, such as grasses, can also decrease virus spread in pasture swards, as can removal of grazing pressure to allow healthy plants to out-compete virus-infected plants thus helping by removing them as a source of infection for further virus to spread healthy plants by aphids (Jones and Ferris 2000).

For the different combinations of virus and plant host within mixed species white clover pasture swards, suppressing virus spread requires an integrated approach combining a range of appropriate control measures. Those available for white clover include avoiding introduction of contact-transmitted viruses (i.e. WSMV) to new pastures on farm machinery or stock moving from older infected pastures, avoiding sowing new pastures in fields close to old infected ones, sowing virus-resistant varieties if available, admixture of susceptible species with pasture species that are non-hosts, well timed applications of newer generation pyrethroid insecticides to kill aphid vectors, and relaxing grazing pressure to (i) allow swards to grow up such that healthy plants shade out more stunted, virus-infected source plants, and (ii) minimise spread by contact-transmitted viruses (Jones 1996; McKirdy and Jones, 1997, 1999; Barbetti and Jones, 1999; Coutts and Jones 2002).

9.6.9 What are the gaps and research needed?

The critical gaps that need addressing in relation to foliar diseases of perennial clovers for southern Australia are as follows:

Survey white clover pastures and other clover perennial pastures cross southern Australia to establish relevant benchmarks for fungal foliar disease and virus disease incidences and define yield impacts.

Define the strains of AMV, WCMV, SCRLV, SCSV and CYVV occurring in perennial pasture clovers across Australia such that available host resistances can be effectively deployed.

Quantify yield losses caused by the most important of the foliar fungal and viral pathogens in grazed monoculture swards of white clover and grazed white clover – grass mixtures.

Define relative resistances of current varieties and near-release breeding genotypes across all perennial clovers to the most important of the foliar and viral pathogens.

Screen perennial clover germplasm and breeding lines to identify sources of natural resistance to AMV and WCMV.

Identify useful sources of natural resistance to the most important of the fungal foliar pathogens of perennial pasture clovers for breeders to utilise.

Ensure seed stocks of new variety releases of perennial pasture clovers are free of seed-borne virus infection

9.7 Annual medics

9.7.1 Soil-borne diseases of annual medics

9.7.2 Introduction

Fungal and nematode soil-borne diseases of annual medics have been recently and comprehensively reviewed by Barbetti et al. (2005) and Barbetti et al. (2006), and while it is not the purpose of this current review to deal in detail across the same territory again, it will include a summary of previous reviews as appropriate for the sake of providing complete coverage in a single document.

9.7.3 Disease symptoms, scope, extent and impact of soil-borne fungal diseases on pasture productivity and fungal, oomycete and nematode pathogens involved

Fungi and oomycete pathogens: Decline of annual medic pastures has been reported throughout the cropping zones of southern Australia (Neal et al. 1997a,b) where their value in crop rotations has been significantly reduced (Bretag 1985). Studies have been conducted to determine decline of annual medic pastures in Australia (Andrew 1963; Kollmorgen 1974; Kellock et al. 1978; Bretag and Kollmorgen 1986; Mebalds 1987; Barbetti 1989c; Bellotti 1998; You et al. 1999, 2000). Kollmorgen (1974), Bretag (1985) and Mebalds (1987) implicated root disease to be critical in this decline. Belotti (1998) undertook a major study to define the relative importance of soil nutrition and root disease in the medic decline syndrome where he conducted 15 experiments during 1996-1997 across three regions of southern Australia, *viz*. Upper Eyre Peninsula, Murray Mallee and Victorian Mallee. He examined medic-based pastures across a range of alkaline Mallee soils and applied treatments of nil, P+Zn, fungicide + nematicide drench (=F+N), and a combination treatment. Results of these investigations showed increases of approximately 25%, 22%, and 62% for the P+Zn, fungicide + nematicide drench, and combination treatments, respectively.

Andrew (1963) implicated damping-off of annual medic seedlings during the early post-emergence period caused by *Pythium* spp., especially in *M. minima*. Kollmorgen (1974) showed that it was mainly *F. avenaceum* that reduced both emergence and dry weight of surviving plants of *M. truncatula*. In south west Western Australia, surveys of annual medic pastures on 116 farms in the grain belt during 1996 and 1997 indicated that one or more soil-borne species of *Pythium*, *Fusarium* and *Phoma* spp. pose a threat to annual medics pastures (You et al. 2000). Considerable research in Australia has targeted *Fusarium avenaceum* as an important pathogen of annual medics (Kollmorgen 1974; Bretag 1985; Bretag and Kollmorgen 1986; Mebalds 1986, 1987; Barbetti 1989c) and likely much more so than on annual clovers. Root rots caused by other pathogens, such as *F. acuminatum, Phoma medicaginis, P. irregulare* and *Rhizoctonia solani*, are also implicated to varying degrees in root disease (Bretag 1985; Bretag and Kollmorgen 1986; Mebalds 1986, 1987; Barbetti 1989c). In Victoria, a comprehensive study undertaken by Bretag (1985) found that *Fusarium* spp. and *Pythium* spp. were important causes of root disease of medics. *Phytophthora clandestina* is also known to attack some annual medics such as *M. truncatula* (Clarke and Greenhalgh 1986; Barbetti 1989c; Greenhalgh 1992), and also, but to a lesser extent, *M. rugosa* and *M. scutellata* (Clarke and Greenhalgh 1986). You et al. (2006) highlighted that this pathogen was present throughout the annual medic growing areas of south-west Western Australia, and this suggests that *P. clandestina* may be of greater significance than first thought. However, the relevance of the many different races of *P. clandestina* to annual medics has still to be investigated.As with their association with annual medic burrs, *Fusarium* species on roots and crowns are cause for additional concern as some *Fusarium* species have been shown to be responsible for the production of mycotoxins deleterious to livestock, both in Australia (Barbetti and Allen 2005, 2007; Tan et al. 2011a,b). The importance to grazing livestock in Australia of burrs, roots, crowns and/or foliage contaminated with *Fusarium* species remains an important situation that is urgently needs to be defined.

Nematode pathogens: The identity (Pung et al. 1988) and the role of plant parasitic nematodes in causing root disease have been investigated for subterranean clover (Ludbrook et al. 1953; Colman 1964; Powell 1971; Pung et al. 1992b), both in their own right (Pung et al. 1991a) and in complexes with fungal pathogens (Pung et al. 1991b), as has the influence upon nematodes of environmental factors (Pung et al. 1992a). As annual medics will continue to be widely sown as a replacement pasture legume in areas historically sown to subterranean clover, it is likely that parasitic nematodes associated with subterranean clover will pose similar threats to annual medics. The incidence and severity of plant parasitic nematodes in annual medic pastures with those in subterranean clover pastures or other legumes grown in rotation need to be compared. Investigation of the occurrence of parasitic nematodes in annual medics in Western Australia (You et al. 1999, 2000) found *Pratylenchus* sp. in medic roots at moderate to high numbers, over 1,000 and 10,000 per g dry weight of roots, at 21 and 14% of 116 sites, respectively. Although these densities are likely to be damaging, they did not conclude that *Pratylenchus* were a key causal factor in the decline of annual medic pastures in Western Australia. A more recent survey by Riley and Kelley (2002) of soil from cropping regions in Western Australia, where annual medics are commonly grown in pasture leys, found *Pratylenchus* in 63% of about 400 sites, with nearly a third at densities that damage roots. The impact of the less common species on annual medics remains to be determined. Researchers in other southern Australian States (Neal et al. 1997b; Hill and Korte 1998) have also noted the potential importance of *Pratylenchus* in medic pastures. While both *Pratylenchus neglectus* and *P. thornei* have been recorded in medics (*e.g.*, Taylor et al. 2000), other *Pratylenchus* species occur in fields used for pastures in southern Australia (Riley and Wouts 2001; Riley and Kelly 2002). Although the host range of these taxa were generally not determined in most of these above studies, the fact that these soils are clearly conducive to parasitic nematodes is of concern. While *Pratylenchus* is common, there are contrasting opinions on their importance. For example, Hutton et al. (1999) indicated that annual medic varieties are mostly regarded as intolerant to *Pratylenchus*. Similarly, consistent with earlier findings (Taylor et al. 2000) and extension advice (Hollaway, 2002), Ballard et al. (2006) have recently determined, for a wider range of annual medic varieties, that medics are moderately resistant and will in fact limit *Pratylenchus* populations in the field. It is noteworthy that while there is a lack of data on impact of *Pratylenchus* on medic growth under field conditions, yield losses of up to 20% in soils with initial populations of about 20 *Pratylenchus*/g have been recorded in South Australia (R. Ballard, unpubl.). Clearly, there is a need to quantify the role and impact of parasitic nematodes on the productivity of annual medics, especially in a field context. No other plant parasitic nematodes have been reported as likely to be a threat for medics other than occasional occurrences of damage from one or more *Meloidogyne* species (MJ Barbetti, unpubl.).

9.7.4 Current Management Strategies – Fungal disease management using chemicals

A range of management strategies have been applied to varying degrees for control of diseases in annual medics (Barbetti 1989a). Fungicides are unlikely to provide effective or cost-effective control of soil-borne diseases of annual medics.

9.7.5 Current Management Strategies - Fungal disease management using cultural practices

<u>Tillage and burning</u>: Tillage can successfully reduce the impact of soil-borne diseases in pasture legumes, as demonstrated for subterranean clover (Barbetti and MacNish, 1984). As many of the root pathogens on subterranean clover and annual medics are common to both genera (Barbetti 1989c), it is likely that similar responses to tillage could be expected for annual medics. However, if any of the existing disease resistances in annual medics are overcome by new pathogen races or more virulent strains, then cultural control strategies will again be needed until resistant varieties become available.

<u>Nutrition</u>: Fertiliser application rates to annual medics pastures have been relatively low, even in areas of highly weathered ancient soils that are inherently infertile (e.g., as in Western Australia). Consequently, such situations require the addition of fertilisers to be productive and it is not surprising that K deficiency frequently reduces pasture production in such areas with sandy soils in particular requiring regular, usually annual, applications of K to maintain profitable production (Bolland et al. 2001). It is frequently the case that plants with good nutritional status are more likely to be better able to resist and/or tolerate diseases (Hannam and Reuter 1987; Wilhelm et al. 1990). In general, plants with an imbalance or a deficiency in one or more nutrition elements can be more susceptible to pathogens, for example, as has been shown between zinc nutritional status of cereals and Rhizoctonia root rot severity (Thongbai et al. 1993; Neate 1994). You et al. (1999) reported strong interactions between the level of nutrition and the severity of diseases in annual medics in Australia. They showed that the severity of these diseases in annual medics were not only partly determined by soil conditions, cultural practices and rainfall, but that more severe root disease was associated with high application of phosphorus fertilisers, soils with relatively high levels of P, NO_3^-, or Fe, and where plant tissues had relatively high levels of total N, K, and S, Cu, Zn, Mn, or NO_3 but inadequate tissue levels of Mg. Their study suggested that the interaction of nutrition and soil-borne pathogens in annual medics may be quite complex.

<u>Rotations</u>: Annual medics form an integral component of cropping rotations because they allow for reductions in weed and disease problems, if grasses and grassy weeds are controlled, in addition to increasing soil N levels for subsequent crops (Walsh et al. 2001), while still providing a feed base for animal production. Rotations not only provide 'disease break' benefits for the annual medic phase itself but more importantly also for the rotational crop species (Barbetti, 1996), such as the reduced level of disease caused by *Rhizoctonia* in wheat in Australia as highlighted by Roget (1995). Certain strains of *Rhizoctonia* may be hosted on a wide range of host plants, rendering rotation less effective than with 'take-all'. Most *Rhizoctonia* spp. (*e.g.,* AG8) can be reduced by soil disturbance (till deep, sow shallow), trash retention (suppression) and nutritional amendments such as Zn.

9.7.6 Current Management Strategies - Fungal disease management using host resistance

While only low levels of resistance to *P. clandestina* has been reported to date in annual medics in Australia, such as for *M. rugosa* and *M. scutellata* (Clarke and Greenhalgh 1986), the existence of high levels of resistance to one or more races of this pathogen in subterranean clover (e.g., You et al. 2005d) suggests that higher levels of resistance could be located in annual medics if widely sought. Although annual medics have been shown to be susceptible to *P. clandestina*, almost no research has been undertaken to identify races of this pathogen in Australia in relation to species of annual medics. While this may be related the proportionately smaller acreages sown to annual medics compared with subterranean clover, it is more likely related to the severe lack of funding for pasture health research in the past decade. With extensive sowings of annual medics, potentially with single dominant gene-based resistance to root pathogens, it is likely with time that a wide spectrum of races could be a problem onannual medics as remains the case with subterranean clover. There is a need for commercial varieties to have effective resistance to the most important soil-borne pathogens in a particular region, if yield losses from such pathogens are to be curtailed.

Only such incorporation of this resistance in commercial varieties could offer a promising long-term management strategy for diseases in annual medics (Barbetti and Nicholas 1997). Several sources of resistance exist overseas that could be usefully employed in Australia. For example, in the United States of America, Haan et al. (2002) identified accessions of *M. polymorpha* with resistance to *Phytophthora medicaginis,* and O'Neill et al. (2003) identified accessions within *M. constricta*, *M. doliata*, *M. heyniana*, *M. laciniata*, *M. lesinsii*, *M. murex*, *M. orbicularis*, *M. praecox*, *M. soleirolii*, and *M. tenoreana* with high level resistance to *Phytophthora medicaginis.* It is clear that all new annual medics released for regions where disease is common, such as across southern Australia, should possess adequate resistance to the major soil-borne pathogens prevalent. In Australia, despite the identification of some sources of host resistance to one or more soil-borne pathogens, breeders are generally yet to deliver this benefit to growers by way of more disease resistant varieties. Even where host resistance has been identified, it is clear that the mechanisms and/or genetic basis of such resistances have not been defined either in Australia or elsewhere. As a precaution, it would be useful for annual medic breeding in Australia to introduce genotypes displaying host resistance to serious soil-borne pathogens of annual medics that are not yet known to be a problem in southern Australia, for example, genotypes with resistance to *Phytophthora medicaginis*.

A review by Tivoli et al. (2006) not only outlined some of the major and/or widespread diseases these necrotrophic pathogens cause on *Medicago* spp., but explored the potential for using the spectrum of necrotrophic pathogen–host interactions, with annual medic as the host plant, to better understand and model pathosystems within the diseases caused by pathogens across forage and grain legume crops. They showed that host resistance clearly offers the best strategy for cost-effective, long-term control of soil-borne (and also foliar) pathogens of annual medics, particularly as useful resistance to a number of these diseases has been identified. Further, they describe how recently and initially, the annual *M. truncatula* has emerged as a more appropriate and agronomically relevant substitute to *Arabidopsis thaliana* as a model plant for legumes, and is proving an excellent model to understand the mechanisms of resistance both to individual pathogens and more generally to most forage and grain legume necrotrophic pathogens. The success and outcome with sourcing resistance in other annual pasture legumes, such as *Trifolium* spp., highlights the value of seeking out new sources of host resistance from the Mediterranean centre of origin, in the same way that has been shown for herbicide resistance (Powles 2001). This is an area of investigation that should allow identification of genotypes with pre- existing multiple resistances to soil-borne (and foliar) pathogens and nematodes and this principle is applicable and equally relevant across the full spectrum of annual and perennial pasture legumes and grasses.

9.7.7 Current Management Strategies - Integrated disease management

Integrated pest management strategies are widely and successfully utilised for some pasture and forage legumes, such as for control of *Kabatiella* on *Trifolium* species across southern Australia (Barbetti 1996), where the application of fungicides in conjunction with using partially resistant varieties and/or application of close grazing provides more benefit than the same or greater amounts of fungicides applied to susceptible varieties, especially where grazing is minimal (Barbetti, 1996). Similarly, integration of different control strategies, including appropriate management strategies, should substantially improve the degree of control for both parasitic nematode and fungal and oomycete soil-borne pathogens of annual medics. Integration of control strategies will remain a key focus until varieties of annual medics with improved host resistance to parasitic nematodes and fungal and oomycete pathogens are available, and, even then, will remain important should such host resistance succumb to more virulent races of one or more these necrotrophic pathogens that affect annual medics.

9.7.8 What are the gaps and research needed?

The critical gaps that need addressing in relation to soil-borne diseases of annual medics for southern Australia are as follows:

- Survey annual medic pastures across southern Australia to establish relevant benchmarks for soil-borne disease incidence and impacts.
- Define relative resistances of current varieties and near-release breeding genotypes across all annual medics to the most important of the soil-borne root pathogens.
- Identify useful sources of resistance to the most important of the soil-borne root pathogens of annual medics for breeders to utilise.

9.8 Foliar diseases of annual medics

9.8.1 Introduction

Fungal foliar diseases of annual medics have been recently reviewed by Barbetti et al. (2006), and while it is not the purpose of this current review to deal in detail across the same territory again, it will include a summary of previous reviews for the sake of providing complete coverage in a single document. Some fungal and viral foliar pathogens, for example *Phoma medicaginis*, are also known to stimulate production of phyto-oestrogenic compounds to high levels that can adversely affect ovulation rates, particularly in sheep but this has not been addressed in detail in this review.

The textbook ‘Viruses of Plants in Australia’ by Buchen-Osmond et al. (1988) lists all viral pathogens then known to infect annual medic species in Australia. Virus diseases of annual medics were included briefly in several early general pasture reviews (Johnstone and Barbetti 1987; Panetta et al. 1993; Jones 1996, 2000). However, viral diseases of annual medics have never been reviewed in detail.

9.8.2 Disease symptoms, scope, extent and impact of fungal foliar diseases on pasture productivity and fungal pathogens involved

There are many fungal foliar pathogens of annual medics and an outline of some of the most important and frequently occurring diseases they cause is as follows:

Phoma black stem: Studies in Western Australia on Phoma black stem disease on annual medics, caused by *Phoma medicaginis,* show that this is one of the most common and important diseases (Barbetti 1983d, 1993, 1995b; Barbetti and Nicholas 1997). It has been found to be a major problem in the second year after seeding in most medic stands, and continues to pose a serious threat to susceptible varieties since its impact was earlier reviewed by Johnstone and Barbetti (1987), especially in rainfall areas of >350 mm per year with long pasture phases between cereal crops, where it causes substantial yield losses (Barbetti and Fang 1991; Barbetti and Nichols 1991b; Barbetti and Nicholas 1997), with reductions in herbage production of up to 16% and seed yield of up to 20% in grazed swards while in ungrazed swards losses of herbage and seed of up to 32% and 53%, respectively have been reported (Barbetti 1992, 1993a). This disease can cause complete defoliation and premature death of very susceptible medics, particularly in lush stands during wet weather (Barbetti 1983d). The effects oftemperature and humidity on the development of Phoma blackstem disease on *Medicago* spp. were investigated by Barbetti (1987c). Disease was greatest at 21/16°, followed by 18/13°C, and least at 15/10°. He found that extending the period of post-inoculation high humidity increased disease severity and the rate of symptom development. Interestingly, *P. medicaginis* was associated with all but 1 of the 16 seed lines tested and the incidence of seed-borne contamination ranged from 0 to 38% (Barbetti (1987c) and this may be a reason for the widespread distribution and damage caused by this pathogen. The importance of this disease in eastern Australia remains to be determined, especially as this same pathogen in known to be an important disease of field peas and isolates from annual medic can cross- infect field peas (Barbetti and Khan 1987).

Leptosphaerulina leaf and stem spot: Leptosphaerulina leaf and stem spot, caused by *Leptosphaerulina trifolii*, commonly occurs wherever annual medics are grown in Australia (Barbetti 1989b, 1995b) and it causes substantial yield losses in susceptible varieties (Barbetti and Nichols 1991b).

Anthracnose: Anthracnose, caused by *Colletotrichum spp.*, is a widespread and very important foliar and crown disease and is caused by *C. trifolii* in Australia (Mackie et al. 1999).

The importance of other diseases on annual medics has not been defined and these include the following:

Pseudopeziza leaf spot: Pseudopeziza leaf spot, caused by *Pseudopeziza medicaginis*, which commonly occurs in Western Australia on *M. polymorpha* var. *brevispina* cv. Circle Valley (Barbetti 1989b) and in eastern Australia (Mackie et al. 1999).

Stemphylium leaf spot: The same applies for Stemphylium leaf spot, caused by *Stemphylium botryosum* and *S. vesicarium*, which has been reported on annual medics in both eastern and Western Australia (Barbetti 1989b; Mackie et al. 1999).

Stagonospora leaf spot: Stagonospora leaf spot, caused by *Stagonospora meliloti*, commonly occurs on *M. polymorpha* var. *brevispina* cv. Circle Valley in Western Australia (Barbetti 1983d, 1989b).

Cercospora: Cercospora (*C. zebrina*) occurs on annual medics in Western Australia but is seldom severe (Barbetti 1985b).

Rusts: Rust (*Uromyces striatus* and/or other *Uromyes* spp.) occur sporadically on annual medics in Australia but little research has been conducted since Walker (1965-1966) first described the rusts of *Medicago* species occurring in Australia more than four decades ago.

9.8.3 Current Management Strategies – Fungal disease management using chemicals

Foliar-applied fungicides potentially offer similar benefits for annual medic pastures (Barbetti 1992) as occurs with annual clovers and a range of management strategies have been applied to varying degrees for control of diseases in annual medics (Barbetti 1989a). Fungicides can be successful in providing economic control on pasture and forage legumes, particularly involving higher return hay or seed production stands, including annual medics (Barbetti 1992).

9.8.4 Current Management Strategies - Fungal disease management using cultural practices

Grazing of annual medic pastures has probably provided substantial disease control of foliar diseases. Similar observations have been noted for diseases on annual medic pastures (Barbetti 1989a; MJ Barbetti, unpubl.). It is apparent from such studies that increased stocking rates and the subsequent closer grazing offer a possible alternative to either fungicidal control or the replacement of susceptiblevarieties of annual medics where disease symptoms occur on foliage. Grazing not only removes much of the infected material, thereby reducing the rate of spread of the pathogen, but new growth can be utilised before being seriously affected. Tillage associated with pasture renovation and burning can probably be helpful by removing infested residues, as shown for foliar diseases of subterranean clover (Beale 1976; Bokor et al. 1978a,b). If resistant types of annual medics replace susceptible varieties, disease control through heavy grazing, burning or tillage will become relatively less important as a management option.

9.8.5 Current Management Strategies - Fungal disease management using host resistance

The utilisation of resistant varieties offers the most cost-effective, long-term control measure for foliar diseases of annual medics and has potential to be spectacularly successful as the main avenue for successful control as it has been for the most important foliar diseases such as *Kabatiella* on *Trifolium* spp. (Barbetti 1996). In particular, these same opportunities exist for at least some diseases, such as Phoma blackstem disease, in annual medics (Barbetti 1993a, 1995b) following on from disease screening programs in Western Australia that have identified good host resistance. However, in Australia, despite the identification of a number of excellent sources of host resistance, breeders are generally yet to deliver this benefit to growers by way of more disease resistant varieties.

Several sources of resistance already exist for certain diseases of annual medics. For example, variation in resistance to stem and leaf disease caused by *Phoma medicaginis* is available in Australia (Barbetti 1987c, 1989a, 1990b, 1995a,b). Variety development programs now need to incorporate available disease resistance, such as those identified for Phoma blackstem disease in Australia (Barbetti 1989a, 1990b, 1993). Such sources of resistance identified in screening programs are useful both as parental materials in breeding and for release of resistant varieties (Barbetti 1995b). Only by incorporation of this resistance in commercial varieties will there be cost-effective and successful long-term management of foliar diseases in annual medics (Barbetti and Nicholas 1997). Barbetti (2007a) determined the differences in host reaction to *Phoma medicaginis* and *Leptosphaerulina trifolii* losses in yield, and consequent effects on herbage quality by inducing the production of the phyto-oestrogen coumestrol. Thirty three varieties and lines in 1993 and 10 varieties in

1995 were evaluated in inoculated field tests (Barbetti 1995a,b). In the 1993 test, a number of genotypes with high levels of resistance to leaf and stem disease caused by *P. medicaginis* and to leaf disease caused by *L. trifolii*, identified. In particular a number of genotypes with very high levels of resistance to stem disease caused by *P. medicaginis* were identified, including *M. sphaerocarpos* GRC5659.4.1 and SAD10069, *M. murex* GRC87.1, GRC707 and GRC708, *M. truncatula* Z771 and *M. solerolii* DZA3180.1, all of which had stem disease scores of ≤ 1.0 (scale 0-10) by the end of the growing season. The 1995 test confirmed the relative responses of 10 varieties (viz. Caliph, Circle Valley, Cyprus, Harbinger AR, Herald, Zodiac, Paraggio, Santiago, Serena and Orion) of annual *Medicago* spp. to leaf and stem disease caused by *P. medicaginis* and to stem disease caused by *L. trifolii*. For stem disease caused by *P. medicaginis* in particular, there was significant positive correlation of the level of disease with the level of coumestrol in stems at the end of the growing season. In contrast, for *L. trifolii*, there was only a weak positive correlation of stem disease with of coumestrol in stems at the end of the growing season. Incorporation of these identified disease resistances into commercial varieties offers a promising avenue not only as a long-term strategy for management of foliar diseases in annual *Medicago* spp., but also as a means of reducing phyto- oestrogen levels in commercial annual *Medicago* spp. pastures in order to minimise the adverse effects of phyto-oestrogens on fertility levels in sheep (Barbetti and Nichols 1991b). As indicated above, in Australia, despite the identification of a significant number of sources of host resistance, breeders are generally yet to deliver this benefit to growers by way of more disease resistant varieties. Even where host resistance has been identified, the mechanisms and/or genetic basis of such resistances have not been defined and this needs attention at least for P. medicaginis.Barbetti (1985b) demonstrated that the annual medic (*M. littoralis, M. truncatula*) varieties screened against *C. zebrina* at that time were susceptible.

9.8.6 Current Management Strategies - Integrated disease management

Integrated pest management strategies for control of foliar diseases should offer annual medics the same potential benefits as obtained for some major foliar pathogens on annual clovers (Barbetti 1996), especially where the application of fungicides in conjunction with using partially resistant varieties and/or application of close grazing provides more benefit than the same or greater amounts of fungicides applied to susceptible varieties, especially where grazing is minimal (Barbetti 1996).

9.8.7 Disease symptoms, scope, extent and impact of viral diseases on pasture productivity and viral pathogens involved

The textbook ‘Viruses of Plants in Australia’ by Buchen-Osmond et al. (1988) lists all viral pathogens then known to infect annual medic species in Australia. Virus diseases of annual medics were included briefly in several early general pasture reviews (Johnstone and Barbetti 1987; Panetta et al. 1993; Jones 1996, 2000). However, viral diseases of annual medics have never been reviewed in detail.

9.8.8 Disease symptoms – viral pathogens

Important viruses

Alfalfa mosaic virus (AMV) is considered of overriding importance on annual medics. Symptoms vary depending on annual medic species, genotype within medic species and AMV isolate. They range from the symptomless infection commonly found in murex medic (*M. murex*) to obvious leaf mottle, leaf deformation and plant stunting commonly found in burr medic (*M. polymorpha*) (Jones and Pathipanawat 1989). In barrel medic (*M. truncatula*), the commonest type of symptom is leaf mottle followed by vein clearing and leaf deformation but other common symptom types included leaf curling, leaf crinkling, pallor, vein banding and plant stunting (Saqib et al. 2011).

Potentially important viruses

Bean yellow mosaic virus (BYMV): BYMV symptoms in plants of annual medics include vein clearing, leaf mottle, leaf pallor, vein banding, leaf deformation, reduction in leaf size and plant stunting. The range of symptom types and severities found vary with medic species, accession and BYMV isolate (Jones and Pathipanawat 1989; Jones 1992; McKirdy and Jones 1995a; Saqib et al. 2011).

Cucumber mosaic virus (CMV): CMV symptoms in plants of annual medics include mottle, leaf deformation and plant stunting. Again, they vary in severity depending upon medic species, accession and CMV isolate (Jones and Pathipanawat 1989; Jones 1992; McKirdy et al. 1994b; Saqib et al. 2011).

Subterranean clover mottle virus (SCMoV): Symptoms of SCMoV infection in artificially-infected barrel medic, burr medic, strand medic, cut leaf medic and disc medic plants include vein clearing, mottle, vein banding, pallor, leaf deformation, reduction in leaf size and plant stunting. However, some other medic species were only infected in inoculated leaves or did not become infected (Wroth and Jones 1992a; Forsu-Nyarko 2002; Saqib et al. 2009)

Viruses occasionally found in medics

BLRV and BWYV: BLRV and BWYV were found in barrel medic weeds in VIC, both associated with purple and chlorotic leaf margins (Freeman and Aftab 2011)

SCSV: SCSV is found in medics in TAS, QLD, NSW and VIC. It causes chlorosis of young leaves, shortening of internodes and plant stunting in naturally SCSV-infected plants of black medic (*Medicago lupulina*), burr medic (*M. polymorpha*) and woolly bur medic (*M. minima*). Inoculated plants of burr medic develop leaf puckering, marginal chlorosis, and severe stunting with a rosette appearance (Buchen- Osmond et al. 1988). It also infects several other medic species which are listed in full under SCSV in the subterranean clover section above.

SCRLV and WCMV: Both are reported infecting burr medic in TAS and QLD respectively (Buchen- Osmond et al. 1988).

Bean common mosaic virus, Lucerne Australian latent virus, Peanut mottle virus and *Watermelon mosaic virus* are also reported to infect annual *Medicago* spp. in Australia by Buchen-Osmond et al. (1988).

9.8.9 Extent and impact of viral diseases on pasture productivity

AMV: The outbreak of AMV in the late 1970s in eastern Australia was followed by an outbreak after 1980 in WA (Buchen-Osmond et. 1988). AMV infection is very common in annual medic introduction, breeding and seed increase plots of varieties, advanced selections and accessions of diverse medic species in Australia. This is because their seed stocks are also frequently AMV-infected: when medic seed lots from
1984-1986 were tested, seed-borne AMV was found in all 12 varieties and 44/50 numbered selections tested. Seed transmission rates ranged from 0.3-74% and exceeded 5% in 33 seed lots. In contrast, when older seed stocks were tested none from 1971-1978 were infected, the oldest infected seed lot being from 1980, suggesting that contamination of the medic breeding program with AMV started at that time (Jones and Pathipanawat 1989). In commercial seed stocks of medic varieties released in 1986, AMV was often found and occurred at incidences up to 26% showing that inadvertent release of AMV- infected seed by breeders had occurred. When medic pastures in WA were surveyed in 1987, AMV was only detected in 3/47 (Jones and Pathipanawat 1989). Shortly after that, when medic pastures in WA were surveyed in 1991-1993, AMV was detected in 9/91 with infection incidences up to 21% (McKirdy and Jones 1995). These low infection levels presumably reflected a predominance of sowing such pastures before 1980 when AMV was absent from the medic program, because once AMV-infected medic seed was sown in pasture plots, annual sward infection was sufficient for it to persist over a 12 year period in successive annual seed harvests (McKirdy and Jones 1995). There is a need for new surveys of medic pastures to establish the consequences of sowing AMV-infected seed inadvertently over many years, as was already done with lucerne pastures (Jones 2004b).

AMV usually causes relatively mild disease symptoms in medics and for this reason is commonly overlooked in annual medic based pastures. However, the losses it causes are considerable. In experiments with sap-inoculated plants growing in pots, AMV decreased herbage and root production (dry weights) of burr medic plants by 30-60%. In spaced plants of burr medic, reductions of about 60% were found in seed yields as well as in herbage and root production (dry weights) (Jones and Pathipanawat 1989). Similar losses were obtained resulting from AMV infection of simulated barrel medic swards (Dall et al. 1989). In mown, first year mini-swards and mown or grazed swards sown with infected seed of burr medic, AMV spread by naturally occurring aphids caused herbage and seed yield losses of up to 32%. Losses of up to 29% occurred in grazed, monoculture swards of burr medic that regenerated after cereals (Jones and Nicholas 1992; Jones 1993). Moreover, AMV decreases nitrogen fixation in burr medic by impairing nodule function and in barrel medic by decreasing nodule numbers (Dall et al. 1989; Wroth et al. 1993). It also increases the content of the phyto-oestrogenic compound coumestrol which causes infertility in sheep (Jones and Ferris 1995). In annually self-regenerating, mixed species pasture swards originally sown with infected medic seed, AMV infection continued to have a major impact on pasture species composition several years after its introduction in sown seed, allowing the proportion of less desirable species such as capeweed to increase at the expense of the medic (Jones and Nicholas 1998). Diminished legume content, and a corresponding increase in undesirable species like capeweed, results in poor feed quality at the critical late autumn early winter ‘feed gap’ period and subsequently during the growing season. It also results in diminished medic seed yields which, when compounding year by year, gradually deplete the seed bank eventually leading to deteriorated, weed-dominated pastures (Jones and Nicholas 1998).

BYMV: BYMV is reported infecting plants of different annual medic species in QLD, SA and WA (Jones 1987, 1988, 1989, 1990; Buchen-Osmond et al. 1988). Infection was found sporadically in breeding and evaluation program plots of several medic species in WA (Jones and Pathipanwat 1989; Pathipanawat et al. 1990). However, no surveys have been undertaken to determine its incidence in commercial medic pastures or seed stocks. BYMV infection diminished herbage and root dry weights by 38-61% in spaced plants of burr medic (Jones 1992).

CMV: CMV is reported infecting plants of different annual medic species in VIC, SA and WA (Garrett 1985; Jones 1987, 1988, 1989, 1990; Buchen-Osmond et al. 1988; Freeman and Aftab 2011). Infection was widespread in medic breeding and evaluation program plots of several pasture and wild naturalised medic species in WA, and CMV-infected plants were often co-infected with AMV (Jones and Pathipanawat 1989; Pathipanawat et al. 1990; McKirdy and Jones 1994b). In spaced plants, CMV infection diminished herbage and root dry weights by 78-90% in murex medic, and by 56-82% in burr medic. It reduced burr medic seed yield by 94% (Jones 1992).

SCMoV: SCMoV has not been reported infecting breeding and evaluation plots of annual medics but infection would not be expected in them because the virus is not vector transmitted but contact- transmitted and spread by grazing animals (e.g. McKirdy et al 1998). Medic pastures have never been examined for presence of SCMoV. Surveys of pastures are required to establish the occurrence of SCMoV, especially with barrel medic, burr medic, strand medic and disc medic. Yield loss information for SCMoV is not available for annual medics.

9.8.10 Aetiology and epidemiology of viral pathogens

AMV: AMV is readily seed-borne in annual medics and is transmitted non-persistently by many aphid species. It was first recorded in Australia in 1945 but surveys in 1966-1975 did not detect it in lucerne pastures in NSW and VIC. The situation changed in the late 1970s when large amounts of seed of aphid-resistant alfalfa varieties heavily infected with AMV were inadvertently imported from the USA, and three legume aphid species which transmit AMV were introduced to Australia, the blue-green aphid (*A. kondoi*), spotted alfalfa aphid (*Therioaphis trifolii*) and pea aphid (*A. pisum*) aphids (Garran and Gibbs 1982; Buchen-Osmond et al. 1988; Hajimorad and Francki 1988). In annual medics in Australia, no infection was found in any of seed lots harvested before 1980, and the virus was first found in medic plots in 1986. Seed transmission rates in medics are often high, e.g. up to 74% in burr medic (Jones and Pathipanawat 1989; Pathipanawat et al 1995). For annually self-regenerating medic pastures in agricultural areas of WA, the main source of AMV is the sown medic seed itself. Sowing virus-infected medic seed into new pastures introduces potent sources of virus distributed at random within the growing sward. Aphid vectors acquire the virus from the seed-infected plants and spread it to healthy medic plants (Jones and Nicholas, 1992, 1998). The blue-green aphid is its most important vector in medic swards and wingless forms walking between intermingling plants in medic swards and forming localised infection patches provide the most important method of spread, spread over distances by winged forms and initiation of new infection foci away from primary infection sources is less frequent (Jones and Nicholas 1998). Other species found in medic pastures that are also likely to play a role in spreading AMV are the spotted alfalfa aphid and cowpea aphid both of which colonise medics, and others that colonise weeds or grasses within mixed-species pasture, e.g. the green-peach aphid colonising broad- leafed weeds such as capeweed, and oat and corn aphids which often colonise grasses (Ridsdill-Smith and Scott 1997; Thackray and Jones 1999; Jones and Nicholas 1988; Jones and Ferris 2000).

Given a suitable soil type for annual medic, the information obtained from long term field experiments suggested that AMV can persist indefinitely in annually regenerating pasture originally sown with infected medic seed. However, infection remained remarkably localised to where the infected seed was originally sown. The virus is also seed-borne in naturalised wild annual medic species, and at least two common annual weeds, flatweed (*Hypochaeris glabra*) and rufous stonecrop (*Crassula decumbens*), so annual weeds can sometimes play a role as secondary virus sources (Jones and Pathipanawat 1989; McKirdy and Jones 1994b; Pathipanawat et al. 1995).

BYMV: BYMV was seed-borne in burr medic, barrel medic, murex medic and *M. aculeata* at seed transmission rates up to 1% (McKirdy and Jones 1995a; Pathipanawat et al. 1995). These results suggest that BYMV can persist over the dry summer period in medic pastures through carry over in dormant medic seeds. Aphid transmission of BYMV in medic pastures has not been studied, but is likely to resemble its spread by aphids in clover pastures (see above).

CMV: CMV was seed-borne in burr medic at seed transmission rates up to 13% (Garrett 1985; McKirdy and Jones 1994b; Pathipanawat et al. 1995). It was also seed-borne at lower levels in murex medic and M. *rigidula* (Pathipanawat et al. 1995). Again, these results suggest that CMV can persist over the dry summer period in medic pastures through carry over in dormant medic seeds. Aphid transmission of CMV in medic pastures has not been studied, but is likely to resemble its spread by aphids in clover pastures (see above).

SCMoV: SCMoV is seed and contact transmitted in subterranean clover, and would be expected to behave this way in annual medic pastures. However, no spread or epidemiological studies have been made with this virus in annual medics.

9.8.11 Current management strategies – viral diseases

AMV: To prevent AMV infection from contributing to declining legume contents of medic pastures, virus- tested seed should always be used when medic is first sown. However, field experiments with plots sown with healthy and infected medic seed which were allowed to regenerate naturally each year over a 6-year period and were grazed, suggested that sowing seed with very low levels of infection will rarely result in a major impact on pasture composition (Jones and Pathipanawat 1989; McKirdy and Jones 1995; Jones and Nicholas 1998). This research resulted showed that a ‘threshold’ of <0.1% infection for medic seed stocks used to sow new pastures was adequate to avoid the risk in later years of economic losses due to decrease in medic feed for livestock resulting from AMV infection (Jones and Nicholas, 1998). A seed testing service for medic seed samples is now available for medic seed stocks prior to sowing. It involves testing 1000 seeds/sample for AMV seed transmission (Jones and Pathipanawat 1989; Jones and Nicholas 1992; McKirdy and Jones 1994; Jones 2000).

In field experiments sown with AMV-infected or healthy seed of burr medic and grazed by sheep, seed- infected plants acted as primary sources for virus spread by naturally occurring aphids. In these experiments, admixture with annual ryegrass (*Lolium rigidum*), a non-host of AMV, and different insecticides were used in attempts to control virus spread. Sowing swards to provide the ratios 1:4 and 1:13 of medic:ryegrass plants diminished AMV spread in medic plants by 23% and 45% respectively.

Applications of organophosphorus (demeton-s-methyl), carbamate (pirimicarb) and newer generation synthetic pyrethroid (alpha-cypermethrin) insecticides, all significantly decreased final AMV incidence. Alpha-cypermethrin was the most effective, suppressing AMV incidence by 87% (2 sprays), 79% (1 late spray) and 65% (1 early spray). Two sprays of demeton-s-methyl decreased incidence by only 36%, while two and 2 weekly applications of pirimicarb diminished it by 29-65% and 35-70% respectively. AMV infection of medic seed harvested decreased by up to 76% in sprayed plots. Insecticide treatment did not prevent winged aphid vectors from landing but numbers of wingless blue-green aphids (*Acyrthosiphon kondoi)* colonising swards were suppressed by up to 92% by spraying with pirimicarb and up to 96% by alpha-cypermethrin. Blue-green aphids were much slower to recover with alpha-cypermethrin than with pirimicarb, the former still significantly diminishing its numbers 35 days after spraying. Greater effectiveness of insecticides in controlling spread of AMV in pasture than has been found previously with non-persistently aphid-transmitted viruses in annual crops seems due to the key role played by wingless aphids as virus vectors. This research shows that application of admixture with grass and application of pyrethroid insecticide can be used to control AMV spread in medic swards (Jones and Ferris 2000).

Pathipaniwat et al. (1996) found three types of resistance to AMV when 75 accessions of button medic (*M. orbicularis*) were inoculated with AMV using aphids. These were extreme resistance in SA15222, systemic hypersensitivity in SA2570, SA9310, SA10282, SA12337 and SA15052, and partial resistance in SA5073, SA13905 and SA17711. Hypersensitivity in SA10282 was controlled by single dominant gene *Nam-1*. However, sap inoculation of plants of 212 accessions in the core collection of barrel medic with AMV did not reveal any sources of AMV resistance (Saqib et al. 2011). There is considerable scope for screening for AMV resistance sources in other annual medic species, and for using *Nam*-1 and any other resistance genes found to breed annual medics with AMV resistance.

In summary, for AMV-infected pastures minimising both their spread during the growing season and their build up in the seed bank can be achieved by sowing healthy seed stocks, cultural methods designed to decrease the carryover of infection between growing seasons (e.g. admixture with grasses) and application of appropriate insecticides to slow AMV spread. Thus, control measures include sowing healthy seed stocks, admixture with grasses and insecticide application to kill aphid vectors (Dall et al. 1989; Jones and Pathipanawat 1989; Jones and Nicholas 1992, 1998; Jones 1996, 2004a).

BYMV: When the accessions in the core collection of barrel medic were inoculated with BYMV most were susceptible but systemic hypersensitive phenotypes developed with isolates LKoj1-NN and LP-1 in plants of 4456, or with LKoj1-NN only in 774, 1526, 4327, 14829, 15268, 22922 and 25654 (Saqib et al. 2011). This suggests that breeding for BYMV resistance is possible in burr medic.

CMV: When the accessions in the core collection of barrel medic were inoculated with CMV most were susceptible but plants of accession 11715 remained uninfected by CMV isolates CP (CMV subgroup 1) and LW (CMV subgroup 2). In addition, plants of 21362 developed systemic hypersensitive phenotypes with CMV CP and LW, while plants of 1526, 2748 and 31443 developed them with CP (Saqib et al. 2011). This suggests that breeding for CMV resistance is possible in burr medic.

SCMoV: When the accessions of the core collection of barrel medic were inoculated with SCMoV, the plants of 42 accessions remained uninfected systemically and so were potentially SCMoV resistant. Accession DZA315.16 developed a localised hypersensitive reaction to

SCMoV determined by a single resistance gene (Saqib et al. 2009). This suggests that breeding for SCMoV resistance is possible in barrel medic.

9.8.12 What are the gaps and research needed?

The critical gaps that need addressing in relation to foliar diseases of annual medics for southern Australia are as follows:

- Survey annual medic pastures across southern Australia to establish relevant benchmarks for foliar fungal disease and viral pathogens of annual medics and obtain yield loss data from grazed swards.
- Define relative resistances of current varieties and near-release breeding genotypes across all annual medics to the most important of the foliar fungal pathogens and viral pathogens.
- Define the strains of AMV, BYMV and CMV occurring in annual medic pastures across Australia such that available host resistances can be effectively deployed.
- For major fungal and viral diseases, define relative resistances of all near-release breeding genotypes across annual pasture medic species, identify useful sources of resistances to these pathogens for breeders to utilize.
- Ensure seed stocks of new variety releases of all annual medics are free of seed-borne virus infection.

9.9 Lucerne

This section focuses lucerne fungal and viral diseases in southern Australia. Considerable research has been conducted on lucerne fungal diseases in tropical pastures but this is outside the terms of reference for this review.

9.9.1 Soil-borne diseases of lucerne

9.9.2 Introduction

Lucerne cultivation in Australia went through a steep decline after the introduction to and establishment in Australia in 1966-1975 of three legume aphid species, the blue-green aphid (*A. kondoi*), spotted alfalfa aphid (*Therioaphis trifolii*) and pea aphid (*A. pisum*) (Garran and Gibbs 1982; Buchen-Osmond et al. 1988; Hajimorad and Francki 1988; Jones 1966, 2004b; Dear et al. 2003; van Leur and Kumari 2011). These introductions resulted in the importation of large amounts of seed of aphid-resistant alfalfa varieties from the USA, but unfortunately the imported seed stocks were contaminated with seed-borne virus infection which resulted in a big upsurge in seed-borne virus epidemics in Australian lucerne stands. Moreover, introduction of the three new legume aphids, which are also virus vectors, will have increased levels of aphid-borne viruses in general including both seed-borne viruses transmitted by aphids and non seed-borne viruses (Garran and Gibbs 1982; Buchen-Osmond et al. 1988; Hajimorad and Francki 1988; Jones 1966, 2004b; van Leur and Kumari 2011). Large-scale virus surveys of lucerne stands and seed stocks have been undertaken in eastern and WA (Garran and Gibbs 1982; Buchen-Osmond et al. 1988; Hajimorad and Francki

1988; Jones 1966, 2004b; van Leur and Kumari 2011). Also, several early studies were undertaken in eastern Australia to identify and characterise other viruses infecting lucerne (e.g. Svenson and Venables 1961; Behncken 1966; Blackstock 1978; Remah et al 1986; Forster et al. 1989; Jones et al. 1989; Forster and Jones 1980; Jones and Foster 1980). The textbook 'Viruses of Plants in Australia' by Buchen-Osmond et al. (1988) listed all viral pathogens then known to infect lucerne species in Australia, and several others are listed in more recent articles (Jones 1996, 2004b; van Leur and Kumari 2011). Lucerne pastures that provide a significant reservoir of virus infection for spread to legume crops, e.g. in NSW (van Leur and Kumari 2011). Virus diseases of lucerne in Australia were included briefly in several early general pasture reviews (Johnstone and Barbetti 1987; Panetta et al. 1993; Jones 1996). However, they have not been reviewed in detail.

The occurrence and relative importance of lucerne fungal diseases in rainfed and irrigated pastures of southern Australia has not been assessed in detail, certainly not in recent decades, especially in relation to the nature and spectrum of fungal pathogens that will replace or add-on to those that are dominant in irrigated pastures. Further, lucerne pastures that provide a green bridge in the Mediterranean summer are likely to attract biotrophic fungal pathogens and may benefit from reductions in inoculum build-up or carry-over that occurs with fungal and oomycete pathogens, especially in Mediterranean environments (Sivasithamparam 1993). Unfortunately, much of the results of work done elsewhere on soil-borne diseases of lucerne, in the USA for instance, cannot be extrapolated to the rainfed environments of southern Australia and work is urgently needed in Australia to address this.

9.9.3 Disease symptoms, scope, extent and impact of fungal foliar diseases on pasture productivity and pathogens involved

9.9.3.1 Soil-borne diseases in eastern Australia

Phytophthora megasperma: Much of the research on lucerne pathology in Australia relates to work done in the north and eastern parts of Australia where summer rainfall is common. For example, Phytophthora root rot, caused by the pathogen *Phytophthora megasperma*, is widely distributed throughout lucerne growing areas in eastern Australia (Anon. 1972; Moore and Butler 1979; Clarke 1981b, 1999a; Gramshaw 1981). In particular, in northern Australia, it is a major disease of lucerne (Musial et al. 2005). Symptoms include wilting and yellowing of individual plants or patches of plants within the stand. Large brown sunken, circular to elongated lesions, consistently originating at the junctions of the tap-root with a lateral root (Marks and Mitchell 1971a; Irwin 1976) are present and eventually the whole of the tap root rots away leaving only an upper stub attached to the crown. Tips of lateral roots are also commonly found to be rotted (Marks and Mitchell 1971a; Irwin 1976). The fungus is able to survive in the soil for many years without the presence of host plants. Young stands are particularly susceptible to root rot and heavy losses can occur if wet conditions prevail especially during establishment year (Purss 1959; Purss 1965; Anon. 1972; Stovold and Rogers 1975). Losses in older stands are normally seen as a premature stand thinning over a number of years (Purss 1959; Moore and Butler 1979) making early resowing into an unaffected area necessary (Stovold 1976b; Stovold and Rogers 1975). In Queensland *P. megasperma* seriously reduces lucerne persistence and productivity in many areas (Purss 1965; Irwin 1974). In New South Wales during wet years root rot becomes important and widespread and severe losses have occurred (Anon. 1972). It is a major factor in stand decline of lucerne growing on wet soils in NSW (Anon. 1975b). For example, in the Hunter Valley and along the Lachlan River the disease is so much of a problem that some stands of Hunter River lucerne fade away in only two or three years (Moore and Butler 1979). Losses in Victoria are generally lower (Clarke 1981b, 1999a). Marks and Mitchell (1971a) studied the penetration and infection of lucerne roots by *P. megasperma* and the pathological anatomy of infected roots. They demonstrated the accumulation of zoospores on the root tips of lucerne around the zone of cell division and cell elongation, and the subsequent invasion of this region resulting in rotting of the root tip. Marks and Mitchell (1971b) also investigated the factors involved with the reaction of lucerne to root rot caused by *P. megasperma* by comparing the response of susceptible and tolerant varieties and selections to infection. They reported the occurrence of a hypersensitive type reaction in the cortical cells of the growing root tip after infection had taken place in resistant varieties. Roots with large-diameter central steles survived better in infested soil. Tolerant selections produced more lateral roots with large diameter central steles than did those susceptible to root rot. Irwin (1976) studied the infection of roots of resistant and susceptible lucerne varieties by zoospores of *P. megasperma*. He demonstrated that zoospores accumulated, encysted, germinated and penetrated roots at two different regions, the region of cell division and cell extension at the root apex and at the junction of a lateral root with the tap root. This explained why tap root lesions consistently originated at the junction of the tap root with a lateral root on plants infected in the field.

Rhizoctonia root canker: Rhizoctonia root canker, caused by the fungus *Rhizoctonia solani*, occurs in all lucerne areas and can cause up to 40% loss of plants (Clarke 1981b). Affected stands appear patchy due to the presence of normal plants growing among stunted and wilting plants. The fungus attacks the base of small lateral roots producing brown to black cankers 1-2 mm up to 10 mm which may eventually girdle the whole root, causing the plant to wilt and die (Clarke 1981b, 1999a). This disease is favoured by periods of high temperature and high soil moisture (Purss 1965), particularly when wet conditions follow a period of hot dry weather (Gramshaw 1981). Excessive rainfall, poor irrigation practice, mechanical damage to roots and crown and unfavourable soil conditions have been associated with disease outbreaks (Clarke 1981b). In addition, *Rhizoctonia solani* also causes a stem blight, a disease most evident in the first or second year after planting in high temperature high moisture conditions (Clarke 1981b). In this disorder, the fungus attacks the buds and young shoots at and below ground level, causing brown lesions. As the infection progresses the fungus grows well into the crown, girdles the stems, and causes leaves to turn yellow and wilt. *Rhizoctonia solani* readily survives for long periods in the soil particularly in infested plant materials.

Stagonospora root and crown rot: Stagonospora root and crown rot, caused by the fungus *Stagonospora meliloti*, occurs in all districts in eastern Australia, but its incidence is relatively low (Clarke 1981b, 1999a). The fungus causes a rotting of the roots and crown but fungus progress in plant tissues is slow.

A characteristic symptom of this disease is the V-shaped front of rotting tissue moving down the root below the crown (Clarke 1981b, 1999a). Orange-red flecks can usually be seen just below the disease front. Eventually the root becomes necrotic and the plant dies.

Acrocalymma root and crown rot: *Acrocalymma medicaginis* root and crown rot commonly observed in Queensland (Irwin et al. 2004) causing red streaking at the extremity of wedge-shaped dry-rotted tissue.

Aphanomyces: *Aphanomyces euteiches* was recorded as possible cause of poor lucerne establishment in Queensland and showed high levels of virulence on lucerne (Abbo and Irwin 1990).

Fusarium root and crown rot: Fusarium root and crown rot, caused by various species of *Fusarium* is present in lucerne throughout both eastern (Clarke 1981b) and Western (Marcley 1970) Australia. Losses may vary from minor to very severe (Clarke 1981b). Symptoms include curling of leaf edges, wilting of foliage and stunted plants with yellow leaves. Crowns and tap roots of affected plants are discoloured from pale brown to black and may also show a red-brown vascular discolouration. Death of affected plants usually occurs in warm dry weather and crowns and tap roots are normally completely rotted by this stage. Excessive moisture and high temperatures are ideal for the fungus to grow and attack the plants (Clarke 1981b). Marcley (1970) investigated lucerne decline in Western Australia and found *F. oxysporum* to be the most frequently isolated *Fusarium* spp. and he demonstrated its pathogenicity to lucerne seedling roots.

Sclerotium rot: Sclerotium rot, caused by the fungus *Sclerotium rolfsii*, is more common in areas of high rainfall and high humidity at temperatures >25^{o}C (Anon. 1972). Young stands are more subject to attack and losses of 5-10% have been reported, but generally losses are not important (Anon. 1972). Symptoms are wilted plants scattered throughout the stand. Affected plants show evidence of rotting of the crown and stems and sometimes also the taproot. White mycelial growth is usually present on the surface of rotted tissues and brown sclerotia also develop. Infected plants are usually killed. Infection originates from soil-borne sclerotia that germinate during moist conditions. Sclerotia may be transported by many agents including machinery, animals, hay and seed (Anon. 1972).

Seedling blight and damping-off: Seedling blight and damping-off, caused by various species of *Pythium*, occurs in all lucerne growing areas where environmental conditions favour disease development. In Queensland seedling blight occasionally occurs when excessively wet conditions follow sowings in cold soils (Purss 1965). In NSW, severe losses are rare but re-sowing is sometimes necessary (Anon. 1972) and, in Victoria, it is a major problem in wet, poorly drained soils (Clarke 1981b). Symptoms are newly sown seed failing to emerge satisfactorily and/or seedlings emerging satisfactorily and then dying soon afterwards. In affected emerged seedlings the basal tissue develops a soft rot causing collapse of seedlings. *Pythium* spp. are ubiquitous soil inhabitants that particularly thrive in wet or waterlogged soils and are probably the most common pathogens involved in damping-off of lucerne in southern Australia.

Pythium root rot: Teakle (1956) described a lucerne root rot in south-eastern Queensland that included symptoms of retarded growth, chlorosis, and progressive defoliation. He reported that lateral rootlets, rhizobial nodules and parts of the tap roots were often rotted. He demonstrated both the association and pathogenicity of *Pythium myriotylum*. He reported only two outbreaks of the disease, both within about the first year of establishment.

Violet root rot: Violet root rot, caused by *Helicobasidium purpureum* is a relatively common disease of lucerne in Queensland (Purss 1965). The disease first appears as small circular dead patches which may enlarge to be many metres across. Affected plants are usually yellow and wilted. The root system is covered with light brown to purple fungal hyphae which may extend from the crown to over 150 mm below the soil surface. The disease is generally not regarded as a serious threat to lucerne production, but it can cause serious losses under certain conditions (Purss 1965). Little is known about it occurrence in southern Australia or about the control of this disease where it does occur (Purss 1965).

9.9.3.2 Soil-borne diseases in Western Australia

In Western Australia, a damping-off disorder as occurs in annual clovers and medics, also sporadically occurs in lucerne plantings there (Barbetti et al. 1989). Damping-off can produce two types of symptoms. The first is a pre-emergence rot, which can be seen as failure of plants to emerge. The other is post- emergence damping-off where plants emerge, but then collapse at ground level. Both symptoms are usually found in patches and the area affected can increase rapidly, particularly during cold wet periods. Damping-off is caused by various species, in particular, one or more species of *Pythium,* but also can be caused by *Fusarium* and *Rhizoctonia*, all of which are common soil inhabitants which generally cause most damage under conditions that are less than ideal for seed germination and seedling growth. No practical control measures are recommended in in Western Australia for this disorder.

In Western Australia, and range of fungal root and crown rot diseases occur (Barbetti et al. 1989). For example, lucerne crowns can become infected by one or more fungal pathogens and rot, particularly if they have been damaged during cutting or too-close grazing. Lucerne roots can be invaded by one or more different fungal pathogens, causing various root rotting conditions. In Western Australia, rotting of roots and crowns can involve invasion by one or more species of the fungal genera *Colletotrichum*, *Fusarium, Phoma, Phytophthora*, *Rhizoctonia, Sclerotinia* and *Stagonospora* (Barbetti et al. 1989).

Wright and Burgess (2002), during a survey a survey of lucerne crops in Western Australia reported that there was often more than one root pathogen infecting individual lucerne stands. The pathogen *Rhizoctonia* sp. was only detected in the roots. The type of *Rhizoctonia* was found to be binucleate which is not the same as the *R. solani* types that cause root diseases in cereal and pulse crops. *Fusarium* spp. were detected across all varieties and agricultural zones. This is a common soil pathogen and causes both root rots and crown rots in dry conditions. The pathogens Sclerotinia sp. and Pythium sp. were both detected in some pastures. These pathogens are generally associated with root rots when conditions are cool and wet. The exotic (to Western Australia) pathogen *Phytophthora megasperma* was not detected in any of the samples. They concluded that root disease pathogens were quite prevalent throughout lucerne pastures in WA wheatbelt.

9.9.3.3 Nematode disorders

The root-knot nematodes (*Meloidogyne* spp.) on lucerne are of economic concern partly for the direct feeding damage they cause, but more importantly for the serious damage they can inflict on susceptible crops grown in rotation with lucerne. In Western Australia, root-not nematode is widespread and roots of infested lucerne plants are smaller and show typical swellings or galls on roots. Infection can cause general stunting of plants and severe yield loss. Root lesion nematodes also affect lucerne and are migratory endoparasites that invade plant roots by forcing their way between or through epidermal and cortical cells and feed on cell sap. Many species of root lesion nematodes are associated with lucerne and two species, *P. penetrans* and *P. neglectus* are economically important. *P. penetrans* is relatively uncommon while *P. neglectus* is widespread in Western Australia (Sharma 2001). The root-feeding nematodes, *Meloidogyne* and *Pratylenchus* spp. are found in Australian lucerne pastures (Stirling and Lodge 2005). Given the wide host range of these nematodes, infestation of lucerne by *Meloidogyne* and *Pratylenchus* could restrict the value of lucerne as a rotational species in ley farming systems. The only economic control measure is by rotation with non-host plants such as grasses.

Stem nematode (*Ditylenchus dipsaci*) is the parasitic nematode of concern in lucerne, and was recognised as early as 1932 (Edwards 1932). Although stem nematode is not a root pathogen, it is a soil-borne organism but survival in soil is not main means of dispersal. Host resistance is the most practical means of control and the SARDI lucerne-breeding program seeks to incorporate resistance in varieties released (www.sardi.sa.gov.au/pages/pastures/breeding_lucerne.htm). The limited research in Australia on parasitic nematodes of lucerne clearly needs to be redressed given the growing interest in using lucerne for it environmental benefits (*e.g.,* Turner and Asseng 2005).

9.9.4 Current Management Strategies – Fungal and oomycete diseases

Rogers et al. (1978) demonstrated that the level of resistance of lucerne to Phytophthora root rot could be increased by cycles of recurrent mass selection in the field. Control is obtained from improving irrigation practices, providing adequate surface drainage (Purss 1959; Clarke 1981b), and use of resistant varieties (Clarke, 1981b, 1999). Irwin (1976) screened a collection of lucerne varieties and strains for resistance to *P. megasperma* both in controlled environment chambers and in a naturally infested field site and identified those with high levels of resistance. Stovold (1976) developed a reliable glasshouse screening technique and demonstrated a significant improvement in the resistance to root rot of certain lucerne lines. Gramshaw et al. (1985) studied the field reaction on North American and Australian lucernes to *P. megasperma* and demonstrated that two Australian lucerne varieties had resistance equal to that found in the best North American lucerne varieties and there is substantial scope to access more resistant genotypes from outside Australia.

Control of Stagonospora crown and root rot involves improving drainage and using 2-3 year rotations between crops (Clarke 1981b). Control of *Fusarium* root and crown rot involves improving drainage and irrigation techniques and using resistant varieties (Clarke 1981b). for Sclerotium rot, complete incorporation of organic matter residues into the soil prior to sowing new areas may help reduce losses in young plants (Anon. 1972). Control of Pythium blight and damping-off is by avoiding sowing during very cold or very wet periods (Anon. 1972), by not irrigating new lucerne too heavily, and by preparing seedbeds early enough to encourage breakdown of infected plant debris before sowing (Purss 1965; Clarke 1981b). Teakle (1956) suggested that correction of unsatisfactory cultural conditions was the most practicable way of reducing losses from Pythium root rot.

In Western Australia the main focus of control for root/crown disorders is to avoid damaging the crown as plants subject to stresses such as over-grazing and trampling by sheep and cattle, mowing too close to the crown, and nutrient imbalances are the ones normally attacked by *Fusarium* spp. (Marcley 1970). Further, Wright and Burgess (2002) suggest the in Western Australia, that if high levels of root rot or crown rot become more prevalent within the stand, it may be necessary to replace the pasture with another crop rather than waiting until the end of the cropping cycle.

9.9.5 What are the gaps and research needed?

The critical gaps that need addressing in relation to soil-borne diseases of lucerne for southern Australia are as follows:

Survey lucerne pastures across southern Australia to establish relevant benchmarks for soil-borne root and crown disease incidence and yield impacts in grazed pasture.

Define relative resistances of current varieties and near-release breeding genotypes across all lucerne to the most important of the soil-borne root and crown pathogens.

Identify useful sources of resistance to the most important of the soil-borne root and crown pathogens of lucerne for breeders to utilise.

9.10 Foliar diseases of lucerne

9.10.1 Introduction

The textbook ‘Viruses of Plants in Australia’ by Buchen-Osmond et al. (1988) listed all viral pathogens then known to infect lucerne species in Australia, and several others are listed in more recent articles (Jones 1996, 2004b; van Leur and Kumari 2011). Virus diseases of lucerne in Australia were included briefly in several early general pasture reviews (Johnstone and Barbetti 1987; Panetta et al. 1993; Jones 1996). However, they have not been reviewed in detail.

While, fungal foliar diseases of lucerne in Australia seem not to have been comprehensively reviewed previously, there is ample extension literature available on these diseases and their management.

9.10.2 Disease symptoms, fungal and bacterial pathogens involved and scope, extent and impact of foliar diseases on pasture productivity

The following is a summary on the importance, symptoms, and biology of common leaf spot, rust, downy mildew, Phoma blackstem and leaf spot, Stemphylium leaf spot, pepper spot and Stagonospora leaf spot, and bacterial wilt in lucerne taken mainly from Barbetti (1983b), Barbetti et al. (1989) and Clarke (1999b), to cover perspectives from both Western and eastern Australia.

Phoma black stem and leaf spot: Phoma black stem and leaf spot is caused by the fungus *Phoma medicaginis*. Spring blackstem and leaf spot is common on lucerne. This disease can affect all above- ground parts of the plant and fungus may extend to the crown and upper root. Numerous small black to dark brown spots develop on the lower leaves, petioles and stems. The irregular shaped lesions on the eaves enlarge, join, and become lighter brown. Affected leaves turn yellow and often wither before falling. Lesions on the stems enlarge and may encircle and blacken large areas near the base of the plant. The fungus also causes a crown and root rot. In Western Australia it occurs on almost all lucerne crops and is frequently the most serious and damaging of the lucerne foliar diseases occurring there (MJ Barbetti, unpubl.). In contrast, in Queensland, the disease has been historically viewed as unimportant although common (Purss 1965). In contrast, this disease appears more damaging in southern Australia where it can affect all above-ground parts of the plant, and the fungus may extend to the crown and upper root (Barbetti 1983b). The fungus overwinters on stems and fallen leaves, and spores produced in spring, while primarily spread by water, are also spread by wind and insects (Clarke 1981a,b, 1999b). Severely infected stands should be cut as soon as possible to reduce leaf loss (Clarke 1981a).

Stemphylium leaf spot: Stemphylium leaf spot, caused by the fungus *Stemphylium botryosum*, is a common disease of lucerne that causes significant defoliation during warm wet periods. The spots on the leaves are oval, slightly sunken, dark brown with lighter centres and they are usually surrounded by a pale yellow halo. Leaflet spots also can appear as more-elongated, irregular lesions with light brown centres and dark brown margins. Older spots may be concentrically ringed, resembling a target. A single lesion can cause a leaflet to yellow and fall. Stems can be blackened. The pathogen survives on infected plant debris, seed and in the soil and is favoured by warm moist conditions. It is dispersed by wind and rain splash and it occurs on lucerne, clovers, and other crop legumes such as lupin and broad bean. In Western Australia, it is generally found on most lucerne stands in Western Australia (MJ Barbetti, unpubl.).

Anthracnose: Anthracnose, caused by the fungus *Colletotrichum trifolii*, causes crown and stem rot (Musial et al. 2005). Anthracnose occurs mainly during warm moist weather (Anon. 1972). In NSW, losses are rarely of any consequence (Anon. 1972) but in Queensland it is considered to be one of the factors responsible for premature thinning of stands (Purss 1965). In particular, it is the more northern regions of Australia where this disease adversely impacts the greatest (Musial et al. 2005). Symptoms are sunken brown to black elongated stem lesions. Lesions are straw coloured with brown borders. Black fungal fruiting bodies (acervuli) develop in the bleached lesions, particularly close to the crown of the plant. On the crown the rotted area is yellow to tan, often with a narrow band of reddish-brown tissue along the border of the advancing rot. Later these rotted crown tissues became bluish black (Clarke 1981b; Gramshaw 1981). Eventually the whole crown and upper tap root may be invaded and plants killed (Gramshaw 1981). The disease is spread by spores produced on dead stems.

Common leaf spot: Common leaf spot caused by the fungus *Pseudopeziza medicaginis*, is a common disease of lucerne. Symptoms include small, circular, dark-brown spots, 2 to 3 mm wide, develop on the leaflets and less frequently elongated spots form on the petioles and stems. These spots usually remain small and eventually a small, disc-shaped, blackish-brown apothecial structure is formed in the centre of each spot. The infected leaves gradually turn yellow cup, turn yellow, then brown before falling to the ground. While this disease does not kill plants, and infrequently causes serious economic losses, disease can be severe during prolonged wet, moderately cool weather when infected plants suffer considerable defoliation, sometimes almost complete defoliation, reducing vigour, hay quality and yield. The fungus carries-over on infected plant debris on the soil surface and subsequent dispersal via infected crop debris is generally wind-blown but also rain splash over short distances. It occurs on both lucerne and medics.

*Rust: C*aused by the fungus *Uromyces striatus* var *medicaginis*, rust is a very common disease of lucerne, with severe outbreaks capable of causing losses through defoliation, and nutritive value. The disease is most damaging on seedlings and in stands shut up for seed or hay. Rust is seen as small, reddish-brown pustules mainly on the under-side of the leaflets and also on petioles and on the stems, especially during warm, humid weather when growth is lush. When severe, leaves wilt and fall prematurely. Spores carry-over in live plant tissue to initiate new infections when conditions are favourable. It is dispersed by wind and rain splash. It occurs on lucerne, medics and sweet clover.

Pepper spot: Pepper spot, caused by the fungus *Leptosphaerulina trifolii*, is one of the most common diseases on lucerne. While this leaf spot primarily affects young leaves, but the fungus also attacks petioles and other above-ground parts. Leaf symptoms vary with the stage of growth, the environment and with the plant's age. Lesions often start as small black spots, but may grow to 3mm wide. The bigger lesions have light brown centres and darker brown borders and are often surrounded by a pale fringe. When severe, this disease can reduce both yield and quality, especially crude protein content. The greatest damage in lucerne occurs on young regrowth after hay cutting. The fungus carries-over in perithecia on infested plant debris, is favoured by cool moist weather, and is dispersed mainly via wind-blown spores from infested crop debris. Pepper spot has been recorded on a wide range of pasture legumes genera and species, including white clover, red clover, subterranean clover and, particularly, on annual medics.

Stagonospora leaf spot: Stagonospora leaf spot, caused by *Stagonospora meliloti*, is generally of minor importance. Stagonospora leaf spots are usually large (5-6 mm diam.) and have a characteristic diffuse margin, a bleached centre and numerous small black/brown fruiting bodies in the infected tissue. Affected leaves soon drop after lesions form. Disease is generally more evident at the shoot tips, have diffuse margins with a bleached centre with small light brown dots, fruiting bodies (pycnidia). Stems and tap roots may also be affected by this disease. This pathogen carries over in pycnidia on infected plant debris and lower stems. It is favoured by warm (20 to 25°C) wet conditions and generally is dispersed under wet conditions, where spores ooze from the fruiting bodies and are spread by irrigation or rain water to neighbouring plants. It is only on lucerne and sweet clover but occasionally also found on other annual and perennial clovers.

Downy mildew: Downy mildew, caused by the fungus *Peronospora trifoliorum* can cause infected seedlings sown in autumn to die or lose vigour. Yield losses are most severe for the first cut. Downy mildew is most common in early spring as it is encouraged by humid conditions and temperatures of 10 - 18^{o}C. Usually the first evidence of downy mildew is a yellow or light green blotching of the leaflets especially at the top of the stem. At the same time, a purplish-grey downy growth develops on the under- side of the affected leaflets and the leaf margins roll downwards. As the disease develops, the leaves may develop a scorched appearance. When young stems are attacked, the result is wilting and death of shoots. The fungus carries-over in infected crown buds and crown shoots. It is dispersed by air movement and occurs on lucerne, white clover and red clover.

Western Australia: In Western Australia, Barbetti (1985b) demonstrated that lucerne was also susceptible *C. zebrina* as occurs in subterranean clover. Wright and Burgess (2002) reported that eight leaf diseases were detected across the pastures sampled during the survey. Of these, Phoma leaf spot (*P. medicaginis*), Stagonospora leaf spot (*S. meliloti*) and Stemphylium leaf spot (*Stemphylium* spp.) were the most common leaf diseases detected. All varieties collected in the survey were susceptible to Stemphylium leaf spot and Phoma leaf spot, and the majority of them were also susceptible to Pepper Spot (*L. trifolii*), Stagonospora leaf spot, and Rust (*U. striatus*). They concluded that leaf diseases were common and prevalent in lucerne pastures and most had more than one disease present. Wright and Burgess (2002) reported charcoal rot (*Macrophomina phaseolina*) in all varieties and in all agricultural zones across Western Australia. This is a common pathogen for legumes and is usually present in older legume crops. It tends to be more prevalent when seasonal conditions are dry than when they are wet. Similarly, Wright and Jones (2003) also reported charcoal rot in all lucerne varieties and in all agricultural zones in Western Australia.

Bacterial wilt: Bacterial wilt can cause serious losses in the eastern States but Western Australia remains free of this disease. Symptoms include yellow-green foliage and stunted plants. Leaf margins are often 'cupped' or curled upwards. Symptoms can appear on single plants scattered throughout the stand or on patches of plants.

9.10.3 Current Management Strategies – Fungal diseases

While Wright and Burgess (220) suggest that while control for these foliar diseases on lucerne is mainly through heavy grazing or removal of the affected hay from the paddock, fungicides can also be useful. Comments in relation to individual diseases are as follows and are largely taken from Clarke (1999b) and/or Barbetti et al. (1989):

Phoma black stem and leaf spot: Keeping stands short, or cutting for hay will help inhibit disease development by lowering humidity within the stand, and reducing the level of infected material which could act as a carry-over source.

Anthracnose: Use of 2-3 year rotations and more resistant varieties are the only control measures. Gramshaw et al. (1985) field screened North American and Australian lucernes for their reaction to *C. trifolii* but no new lines with increased resistance were found.

Pepper Spot: Foliar fungicide sprays can be used in some states, but may not be economic unless they are applied to high value stands.

Common Leaf Spot: If a stand is to be cut for hay, this should be done as soon as possible to minimise losses from leaf drop and build-up of spore numbers. Fungicide sprays have been effective in controlling the disease in the USA, but there are no chemicals registered for such use in Australia.

Stemphylium leaf spot: While foliar fungicide sprays have been registered in some Australian states, usually no control measures are necessary. Hay crops should be harvested as early as possible to reduce losses through leaf fall.

Stagonospora leaf spot: Due to the minor importance of this disease, no specific control measures are recommended. There are no resistant varieties.

Downy mildew: Keeping stands short, or cutting for hay will help inhibit disease development by lowering humidity within the stand, and reducing the level of infected material which could act as a carry-over source.

Rust: Graze or cut for hay to reduce losses and humidity within the stand. This practice also removes rust inoculum from the paddock. Some varieties are more resistant than others to rust infection.

9.10.4 Disease symptoms, viral pathogens involved and scope, extent and impact of foliar diseases on pasture productivity

Infection with AMV is widespread in lucerne pastures and seed stocks in Australia and causes significant herbage and seed production losses (Garran and Gibbs 1982; Johnstone and Barbetti 1987; Jones 1996, 2004b; van Leeur and Kumari 2011). However, the full distribution and importance of the many other viruses infecting lucerne in Australian pastures has yet to be determined.

9.10.5 Disease Symptoms – viral diseases

Most important viruses

Alfalfa mosaic virus (AMV*):* Symptoms of infection are a light green or yellow mottle that may be accompanied by stunting and leaf deformation. Symptoms fade under hot conditions and may not be obvious in dense swards because healthy plants tend to grow over the top of severely affected infected ones (Barbetti et al. 1989).

Potentially important viruses for lucerne

Bean leaf roll virus (BLRV): The infection with BLRV reported in surveys of Australian lucerne pastures was not associated with obvious symptoms (Jones 2004b; van leur and Kumari 2911). Symptomless BLRV infection was also reported in lucerne in Europe (Cockbain and Gibbs 1973).

Beet western yellows virus (BWYV): The infection with BWYV reported in surveys of WA lucerne pastures was not associated with symptoms (Jones 2004b).

Cucumber mosaic virus (CMV): CMV causes an obvious yellowish mosaic and reduction in leaf size in lucerne (Aftab and Freeman 2005).

Lucerne Australian latent virus (LALV): LALV causes symptomless infection in lucerne (Blackstock 1978; Jones et al. 1979).

Lucerne Australian symptomless virus (LASV): LASV causes symptomless infection in lucerne (Remah et al. 1986).

Lucerne transient streak virus (LTSV): Infection of lucerne plants with LTSV causes chlorotic streaking along lateral leaf veins which is sometimes associated leaf distortion. Symptoms may persist but are often transient or absent (Blackstock 1978; Forster and Jones 1979, 1980).

Subterranean clover red leaf virus (SCRLV): The infection with SCRLV reported in surveys of WA lucerne pastures was not associated with symptoms (Jones 2004b).

Unknown luteovirus: In a survey of 31 lucerne pastures in WA in 2001, infection with a luteovirus which was not identified as BLRV, BWYV or SCRLV was detected in four of the stands at incidences up to 5% (Jones 2004b). Similarly, in a survey of 25 lucerne stands in northern NSW in 2009-2010, infection with an additional luteovirus which was not identified as BLRV, BWYV or SCRLV was detected in several of the stands (van Leur and Kumari 2011). No symptoms were associated with this infection in either survey.

Viruses occasionally found in lucerne pastures

Bean yellow mosaic virus (BYMV): BYMV is reported from lucerne in WA (Buchen-Osmond et al 1988).

Red clover necrotic mosaic virus (RCNMV): RCNMV has been reported infecting lucerne in Victoria (Lynes and Teakle 1981). It causes stunting of infected lucerne plants growing under cool conditions (Buchen-Osmond et al 1988).

White clover mosaic virus (WCMV): WCMV is reported infecting lucerne in QLD and WA (McLean and Price 1984; Buchen-Osmond et al. 1988).

9.10.6 Extent and impact of viral diseases on lucerene pasture productivity

AMV: AMV infects lucerne growing as weeds throughout Australia (Svenson and Venables 1961; Garran and Gibbs 1982; Johnstone and Barbetti 1987; Buchen-Osmond 1988; Hajimorad and Francki 1988; Jones 1996, 2004b; Aftab and Freeman 2011; van Leur and Kumari 2011). Forty years ago AMV was rarely found infecting lucerne pastures in Australia (Svenson and Venables 1961). However, in 1966- 1975 three lucerne aphids - the blue-green aphid (*A. kondoi*), spotted alfalfa aphid (*Therioaphis trifolii*) and pea aphid (*A. pisum*) - were introduced and became widely established (Garran and Gibbs 1982; Johnstone and Barbetti 1987; Jones 1996). These introductions resulted in the importation of large amounts of seed of aphid-resistant lucerne varieties from the USA, but unfortunately many of the imported seed stocks were contaminated with seed-borne AMV infection which resulted in a big upsurge in AMV epidemics in Australian lucerne stands (Garran and Gibbs 1982; Buchen-Osmond et al. 1988; Hajimorad and Francki 1988; Jones 1966, 2004b; van Leur and Kumari 2011).

Virus surveys of lucerne pastures have been undertaken in eastern Australia (Garran and Gibbs 1982; Hajimorad and Francki 1988; van Leur and Kumari 2011), and of both pastures and seed stocks of lucerne in WA (Jones 2004b). In 1981, Garran and Gibbs (1982) surveyed 35 lucerne stands and test plots in the ACT and NSW, and detected AMV in 24 of them at incidences of 25 to >55%. They also detected AMV in seven of 10 commercial lucerne seed stocks, eight of which had just been imported from the USA; AMV incidences in seed lots were 0.4-2%. Hajimorad and Francki (1988) detected AMV in 125/170 leaf samples of lucerne from 13 sites in SA. Alberts (1995) found AMV infection occurring commonly in seed stocks of lucerne from SA. In 2001, Jones (2000b) surveyed 31 three-year-old lucerne pastures in WA and AMV was found in 30 of them. Pastures in high, medium and low rainfall zones were all infected. Incidences of AMV within individual infected pastures were high, with 50-98% of plants infected in 20 of them and only 3 having <10% infection. In addition to various variety mixtures, at least 8 different individual lucerne varieties were AMV-infected. These AMV infection levels were considerably higher than those reported earlier by Garran and Gibbs (1982) suggesting that infection with AMV had become much more widespread. In tests on seed samples from 26 commercial seed stocks of lucerne from eastern Australia (especially SA) to be sown in south-west Australia in 2001, infection with AMV was found in 21 of them. Incidences of infection within individual affected seed samples were 0.1-4% and included at least 11 different seed-infected lucerne varieties. In

NSW in 2009-2010, van Leur and Kumari (2011) surveyed 25 lucerne stands and found AMV in 24 of them at incidences up to 91%.

The detrimental effect caused by AMV on productivity of lucerne pastures has been studied widely overseas, with substantial herbage yield losses reported and infection also shown to diminish nodulation and nitrogen fixation (e.g. Gibbs 1962; Frosheiser 1969; Crill et al. 1970; Hiruki and Miczynski 1978; Tu and Holmes 1980; Bailiss and Ollennu 1986; Ohki et al. 1986). Whether similar yield losses also occur in lucerne pastures growing in dryland agricultural conditions has not been determined. However, extensive studies on losses caused by AMV infection in pastures of a related species, annual burr medic (*Medicago polymorpha*), suggest that this is likely. In this species, AMV infection diminishes herbage and seed yields, nitrogen fixation, pasture persistence and the proportion of legume growing in mixtures with other species (Jones and Nicholas 1998; Wroth et al. 1993; Jones 1996; Jones and Ferris 2000). It also stimulates phyto-oestrogen production, which, when in the diet, causes infertility and reproductive disorders in farm animals (Jones and Ferris 2001; Jones 2004b). Widespread infection in lucerne stands, and their frequent colonisation by aphid vectors, is cause for concern not only because of likely virus- induced production losses in lucerne itself but also because they provide virus infection reservoirs for spread to nearby grain legume crops and annual legume pastures (van Leur and Kumari 2011).

BLRV: BLRV has been found infecting lucerne in NSW, VIC and WA (Jones 2004b; Freeman and Aftab 2011; Van Leur and Kumari 2011). In a 2001 survey of 31 lucerne pastures in WA, a 2% infection incidence with BLRV was detected in one pasture, and a 5% BLRV/SCRLV incidence in another (Jones 200b). In a survey of 25 lucerne stands in northern NSW in 2009-20010, half those surveyed were infected with BLRV at variable levels, with infection incidences of up to 33% infection (van Leur and Kumari 2011). Yellow leaves were associated with a BLRV infection in weed lucerne in VIC (Freeman and Aftab 2011). No yield loss data are available for this virus in lucerne. Because BLRV is one of the most damaging viruses to food legume crops in Australia (van leur and Kumari 2011), its appearance is great cause for concern for food legume growers who also plant lucerne.

BWYV: BWYV has been found infecting lucerne in TAS, VIC and WA (Buchen-Osmond et al.1988; Jones 2004b; Freeman and Aftab 2011). In a 2001 survey of 31 lucerne pastures in WA, 1% BWYV incidence was found in four pastures (Jones 200b). No BWYV was detected in a survey of 25 lucerne stands in northern NSW in 2009-2010 (van Leur and Kumari 2011). No yield loss data are available for this virus in lucerne.

CMV: CMV is reported infecting lucerne in SA (Buchen-Osmond et al. 1988). In 2000-2001, tests on seedlings grown from 26 commercial seed samples of lucerne from eastern Australia (mostly SA), detected CMV in three samples at incidences of 0.1-0.3%. However, no CMV was detected in a 2001 survey of 31 lucerne pastures in WA (Jones 2004b) or in a survey of 25 lucerne stands in northern NSW in 2009-2010 (van Leur and Kumari 2011). No yield loss data are available for this virus in lucerne.

LALV: LALV has been found infecting lucerne in VIC (Buchen-Osmond et al.1988). It is reported to occur commonly in lucerne and is probably widespread in lucerne stands in Australia (Jones et al. 1979; Johnstone and Barbetti 1987). Surveys to determine its incidence in lucerne pastures have not been undertaken. No yield loss data are available for this virus in lucerne. Should mixed infection with AMV or other viruses occur frequently such losses are likely to be considerable.

LASV: LASV has been found infecting lucerne in VIC (Buchen-Osmond et al.1988). Surveys to determine its incidence in lucerne pastures have not been undertaken. No yield loss data are available for this virus in lucerne.

LTSV: LTSV has been found infecting lucerne in TAS and VIC (Buchen-Osmond et al.1988). Surveys to determine its incidence in lucerne pastures have not been undertaken. It reduced dry matter production in field plots by 18% (Blackstock 1978).

SCRLV: In a 2001 survey of 31 lucerne pastures in WA, a 2% infection incidence with SCRLV was detected in one pasture, and a 5% BLRV/SCRLV incidence in another (Jones 200b). No SCRLV was detected in a survey of 25 lucerne stands in northern NSW in 2009-2010 (van Leur and Kumari 2011). No yield loss data are available for this virus in lucerne.

9.10.7 Aetiology and Epidemiology – Virus Diseases

AMV: AMV is seed-borne in lucerne and is transmitted non-persistently by aphids (Stuteville and Erwin 1990). Aphids that colonise lucerne in south-east Australia and act as virus vectors there include blue- green aphid *(Acyrthosiphon kondoi*), pea aphid *(A. pisum*) and spotted alfalfa aphid (*Therioaphis trifolii*) (Garran and Gibbs 1982). In WA lucerne pastures, the principal aphid vector species found was blue- green aphid but cowpea aphid (*Aphis craccivora*) and pea aphid also occurred (Jones 2004b). The survey of 26 commercial seed stocks of lucerne imported from the east of the continent for sowing in WA in 2001 revealed that most were infected with AMV. The consequence of importing such infected seed stocks is that they will introduce infection sources when sown within new lucerne pastures thereby making virus epidemics likely once the seed germinates and aphid vectors become active. The large incidences of AMV infection found in the survey of 3-year-old pastures in all rainfall zones of the 'grainbelt' suggested that this is happening (Jones2004b). Aphid vectors acquire the virus from the seed- infected plants and spread it to healthy lucerne plants. Wingless viruliferous aphids walking between intermingling lucerne plants will form localised infection patches and spread over distances by winged aphids will initiate new infection foci away from primary seed-infected plant sources. Van Leur and Kumari (2011) found clustering of AMV-infected plants which they suggested might indicate localised colonisation by aphid vectors as an alternative to localised spread around initial infection foci consisting of seed-infected plants. In weedy lucerne stands, other aphid species found in lucerne pastures that are also likely to play a role in spreading AMV are ones that colonise weeds or grasses within mixed-species pasture, e.g. the green-peach aphid colonising broad-leafed weeds such as capeweed, and oat and corn aphids which often colonise grasses (see above).

Although seed-infected lucerne plants constitute the primary virus source for infection of lucerne stands with AMV, the virus is also seed-borne in naturalised wild annual medic species, and at least two common annual weeds, flatweed (*Hypochaeris glabra*) and rufous stonecrop (*Crassula decumbens*), so annual weeds can sometimes play a role a role as primary sources in addition to the principle initial virus source that results from sowing AMV-infected seed), and this additional AMV may be important in newly planted stands (see above).

Once AMV achieves very high incidences in lucerne stands these are unlikely to decline in subsequent years because, even if the tops die off and the roots remain during the hot, dry summer period, their roots are still AMV-infected and infected plants will grow from them.

Lucerne stands differ from self- regenerating annual medic swards in this respect because the annual epidemic does not have to start off from a low base (the new seed-infected plants) every year.

CMV: There has been no research on the epidemiology of CMV in lucerne stands in Australia, but since it resembles AMV in being non-persistently aphid-borne and seed-borne in lucerne a similar scenario would be likely to occur.

LALV, LASV, LTSV: LALV is seed-borne in lucerne (Taylor and Smith 1971; Blackstock 1978; Jones et al. 1979), but its vector is unknown. Studies to find whether it is pollen-borne in lucerne and identify its vector are needed. It is not known whether LASV is seed-borne in lucerne or what its vector is (Remah et al. 1986). The vector of LTSV is unknown and seed transmission in lucerne is not reported (Forster et al. 1979; Jones et al. 1979). Studies on the epidemiology of these three viruses in lucerne pastures are needed as they are potentially important, particularly when present in multiple mixed infection with AMV leading to greater losses in lucerne stands.

BLRV, BWYV and SCRLV: Luteoviruses like BLRV, BWYV and SCRLV are persistently aphid-borne but not seed-borne. The pea aphid is the most important vector for BLRV and SCRLV (Jones 2004b). BLRV is also transmitted by the green peach aphid and potato aphid, and SCRLV by the foxglove aphid and potato aphid (Kellock 1971; Johnstone 1978; Johnstone and Patten 1981; Helms et al. 1983; Ashby 1984). For BWYV, the green peach aphid is its most important vector but other vectors may play a role in spreading it in lucerne pastures including the cowpea aphid, foxglove aphid and potato aphid. The introduction into Australia of the pea aphid would probably have increased levels of BLRV and SCRLV in lucerne stands; in addition to increasing AMV incidences (see above).

Luteoviruses require a continuous ‘green bridge’ of susceptible plant material to survive dry summer conditions. Increased sowing of lucerne is therefore likely to increase incidences of luteoviruses through accumulation of infections year-after year in its stands. Weed reservoirs of BLRV infection in weeds in Australia require investigation, although infected pulse crops may also act as sources of infection for lucerne in NSW (e.g. Salam et al. 2011). Reservoirs of SCRLV infection are found in certain species of weeds, including storksbills (*Erodium* spp.) and docks (*Rumex* spp.) (Johnstone et al. 1884a; Johnstone and Duffus 1984) and these can act as sources for spread by aphids to lucerne stands. Detection of BWYV in lucerne stands is not unexpected as it has a very wide host range, including common weeds such as wild radish (*Raphanus raphinistrum*) as well as canola and grain legume crops (Coutts and Jones 2000; Latham and Jones 2001b; Coutts et al. 2006; Freeman and Aftab 2011).

9.10.8 Current management strategies – virus diseases

Reduction of aphid populations by cutting for hay or silage and insecticide application should help limit spread of AMV and other aphid-borne viruses in lucerne stands being grown for feed or seed production (e.g. Jones 1996; Jones and Ferris 2000). In contrast, limiting virus spread by mixed planting with a non-host is unlikely to be effective because not only is the proportion of grass required too high (Jones and Ferris 2000) but also virus epidemics can continue all-year-round. Manipulation of grazing pressure to minimise the internal virus source was ineffective at diminishing AMV spread within medic swards (Jones and Nicholas 1992) and would therefore probably be ineffective with lucerne. Resistance to aphids may help to decrease spread of aphid-borne viruses somewhat, so, wherever possible, lucerne varieties with this trait should be sown.

AMV: It is important to minimise not only any productivity losses caused by virus infection in lucerne stands but also virus spread from them to nearby grain legume crops and annual legume pastures. The cornerstone of any strategy to control AMV in lucerne stands is to sow new pastures with seed stocks that are as healthy as possible. For this, routine testing for AMV in pasture seed stocks normally involves1000 seeds/sample (Jones 2000). With AMV infection in burr medic swards, studies over 6 years showed that sowing seed with low amounts of infection rarely resulted in substantial virus spread over distance or a major impact on pasture composition. A ‘threshold’ of <0.1% infection for medic seed stocks used to sow new pastures was adequate to avoid the risk in later years of economic losses due to AMV (Jones 2000). Similar ‘threshold’ studies have not been done with lucerne, in which low levels of seed infection would be expected to result in higher infection incidences because epidemics continue all- year-round. However, a ‘threshold’ of <0.1% AMV would still avoid sowing seed stocks with substantial infection, which would be the ones likely to generate major epidemics quickly (Jones 1980b).

Lucerne seed producers have a ‘duty of care’ responsibility to avoid selling diseased seed. To help them achieve this, regular testing of leaf samples collected from older ‘seed increase’ stands and their replacement as needed by new stands sown with healthy seed would be advisable. Also, if ‘seed increase’ stands of lucerne were sown with healthy seed at locations well isolated from previous lucerne or medic stands, they would be likely to avoid developing large-scale AMV infection for some time. Without action to improve the health status of lucerne seed stocks in eastern Australia, a lucerne seed industry is needed in WA to ensure that sufficient healthy stocks are available locally (Jones 1980b).

AMV-resistant lucerne varieties that are effective against some but not all strains of AMV are available overseas (Stuteville and Erwin 1990). They should be evaluated for adaptation to Australian conditions and breeding for AMV resistance in local lucerne improvement programs should be encouraged (Jones 2004b).

LALV, LASV, LTSV: No control measures against LALV, LASV or LTSV have been studied. Because of the compounding effect of multiple virus infection in plants, their common presence with AMV is likely to depress yields regardless of presence or absence of symptom expression. Sowing healthy seed stocks is an obvious approach to explore with LALV. Information on vectors or contact transmission and on epidemiology would help in devising control measures against all three of them.

BLRV, BWYV, SCRLV: Introduction of luteoviruses to perennial pastures cannot be controlled by seed testing. However, because they are transmitted persistently by aphids, spread can be suppressed readily by applying insecticides (e.g. McKirdy and Jones 1996).

9.10.9 What are the gaps and research needed?

The critical gaps that need addressing in relation to foliar diseases of lucerne for southern Australia are as follows:

Survey lucerne stands across southern Australia to establish relevant benchmarks for foliar fungal and viral pathogens and obtain yield loss data from grazed swards.

Define relative resistances of current varieties and near-release breeding genotypes of lucerne to the most important of the foliar fungal and viral pathogens.

Define the strains of the most important viruses occurring in lucerne stands across Australia such that available host resistances can be effectively deployed.

For major fungal and viral diseases, define relative resistances of all near-release breeding genotypes of lucerne, identify useful sources of resistances to these pathogens for breeders to utilize.

Ensure seed stocks of new variety releases of lucerne are free of seed-borne virus infection.

9.11 Other legumes

9.11.1 Soil-borne diseases of other legumes

9.11.2 Introduction

There has been little or no attempt to determine the important soil-borne root pathogens of other legume genera, such as *Ornithopus*, *Hedysarum* and *Biserrula*, let alone attempt to accurately define their impact or their relevance to rotational species. This is despite the obvious potential for significant areas of these and several other genera and/or species of pasture legumes.

9.11.3 Disease symptoms, scope, extent and impact of foliar diseases on pasture productivity and fungal pathogens involved and current management strategies

There has been some work on *Rhizoctonia* attacking ‘other’ pasture legumes in Western Australia (You et al. 2008). Their study aimed firstly to determine whether the pathogenicity on pasture legumes of strains of *Rhizoctonia solani* sourced from lupins and cereals (common crops in rotation with pastures) is associated with increased incidence of root rots in pasture legumes in the disease-conducive sandy soils of the Mediterranean regions of southern Australia. Their second aim was to determine sources of resistance among newly introduced pasture legumes to *R. solani* strains originating from rotational crops as this would reduce the impact of disease in the pasture phase. Fifteen pasture legume genotypes were assessed for their resistance/susceptibility to five different zymogram groups (ZG) of the root rot pathogen *R. solani* under glasshouse conditions. Of the *R. solani* groups tested, ZG1–5 and ZG1–4 (both known to be pathogenic on cereals and legumes) overall, caused the most severe root disease across the genotypes tested, significantly more than ZG6 (known to be pathogenic on legumes), in turn significantly >ZG4 (known to be pathogenic on legumes) which in turn was >ZG11 (known to be pathogenic on legumes including tropical species). Of particular relevance to this section were the results showing that *Ornithopus sativus* cvs Cadiz and Margurita, showed a significant level of resistance to root rot caused by *R. solani* ZG11 (root disease scores ≤1.2 on a 1–3 scale where 3=maximum disease severity) while *O. sativus* cvs Cadiz and Erica showed a significant level of resistance to root rot caused by *R. solani* ZG4 (scores ≤1.2). *O.*

compressus cvs Charano and Frontier, *O. sativus* cv. Erica showed some useful resistance to root rot caused by *R. solani* ZG6 (scores ≤1.8). These genotypes with resistance may also serve as useful sources of resistance in pasture legume breeding programs and also could potentially be exploited directly into areas where other rotation crops are affected by these *R. solani* strains. However, none of the tested genotypes showed useful resistance to *R. solani* ZG1–4 (scores ≥2.0) or ZG1–5 (scores ≥2.5). This study demonstrates the relative potential of the various *R. solani* ZG strains, and particularly ZG1–4, ZG1–5, ZG4 and ZG6 to attack ‘alternative new’ legume pastures and pose a significant threat to development of such pasture species.

Li et al. (2009a) screened a single genotype of each of *Biserrula pelecinus, Hedysarum coronarium, Ornithopus compressus* and *O. sativus* under controlled environment conditions for resistance to root disease caused by the most pathogenic race of *P. clandestina* occurring in Australia, viz. race 177. This was the first time these particular genera/species had been screened for their response to *P. clandestina*. The root disease caused by *P. clandestina* is the first report of susceptibility to this pathogen for *B. pelecinus*, *H. coronarium* and *O. sativus*. *O. compressus* showed no disease in either test, *O. sativus* showed a disease score of ≤0.7 across both screening tests. *H. coronarium* was susceptible with a disease score of ≤2.7 across 2 separate screening tests, while *B. pelecinus* was highly susceptible with disease scores of 3.5 and 4.5 in these tests. The high levels of resistance identified against *P. clandestina* are useful sources of resistance that can be exploited commercially, either directly to minimise damage from this disease or as parents in breeding programs to develop varieties within the genera/species tested with improved resistance to this highly pathogenic and widely distributed race of *P. clandestina*.

9.11.4 What are the gaps and research needed?

The critical gaps that need addressing in relation to soil-borne diseases of other legumes for southern Australia are as follows:

> Survey pastures of alternative pasture legume species across southern Australia to establish relevant benchmarks for soil-borne root and crown disease incidence and yield impacts in grazed swards.

> Define relative resistances of current varieties and near-release breeding genotypes across all alternative pasture legumes to the most important of the soil-borne root and crown pathogens.

> Define relative resistances of all near-release breeding genotypes across all major alternative pasture species, and identify useful sources of resistance to the most important of the soil-borne root and crown pathogens of ‘other’ legumes for breeders to utilise.

9.12 Foliar diseases of other legumes

9.12.1 Fungal disease symptoms, scope, extent and impact of fungal foliar diseases on pasture productivity and pathogens involved and current management strategies

While it is known that *Ornithopus* can be affected by brown leaf spot (*Pleochaeta setosa*) and *Hedysarum* can be affected by (*Kabatiella caulivora*) (MJ Barbetti, unpubl.), there has been little or no attempt to determine the important foliar pathogens of 'other' legume genera, such as *Ornithopus*, *Hedysarum* and *Biserrula*, let alone attempt to accurately define their impact or their relevance to rotational species. Barbetti (1985b) demonstrated that serradella (*O. compressus*) was resistant to *C. zebrina* as occurs in subterranean clover pastures in Western Australia.

9.12.2 Viral disease symptoms, scope, extent and impact of viral foliar diseases on pasture productivity and pathogens involved

Occurrence of AMV, BYMV and CMV in experimental evaluation and seed increase plots of other pasture legumes was examined over seven years in WA. In 1994 CMV was found in *Biserulla pelecinus* plots. In 1996, BYMV was detected in plots of *B. pelecinus*, and both yellow and pink serradella. In 1997-1998, plots of grass *Lathyrus* spp. (grass pea, *L. cicera*, *L. ochrus, L. sativus*), *Biserrula pelecinus* and *Trigonella balansae* were infected with BYMV at most sites and showed obvious plant symptoms. However, little BYMV infection was found in pink or yellow serradella (McKirdy et al. 1988a; Jones 1999). *B. pelecinus, T. balansae*, *Lathyrus* spp. and pink seradella all developed very severe BYMV symptoms.
In 1999 plots, *T. balansae* was again infected with BYMV. In 2000, a *Melilotus officinalis* plot was infected with AMV, CMV and BYMV. In 2001, both BYMV and AMV were detected in plots of *T. balansae*, BYMV in *B. pelecinus* and CMV in pink serradella. BYMV was seed-borne in *T. balansae*.

When exposed to BYMV infection in replicated single row plots, sulla (*Hedysarum coronarium*) did not become infected, pink serradella was relatively resistant to infection but infected plants of it developed severe plant symptoms, *T. balansae* and *Lathyrus sativus* were susceptible and their plants developed moderate symptoms, and both *Biserrula pelecinus*, *L. cicera* and *L. ochrus* were highly susceptible and developed very severe symptoms. BYMV was seed-borne in *L. cicera* (0.1%, and *L. sativus* (0.2%) (McKirdy et al. 2000). In an earlier study of BYMV infection in replicated single row plots, yellow serradella developed severe symptoms but was relatively resistant to infection (McKirdy and Jones 1995a).

When exposed to CMV infection in replicated single row plots, pink serradella and *Lathyrus* spp. (*L. ochrus, L. sativus*), did not become infected with it. Sulla, *T. balansae* and *B. pelecinus* all became infected with CMV. *T. balansae* and *B. pelicinus* were moderately resistant and became infected without symptoms. In glasshouse inoculations, pink serradella remained uninfected with CMV but *L. ochrus* and *L. sativus* became infected. Infected sulla plants developed severe symptoms but infected *L. sativus* plants developed mild symptoms while infection was symptomless in *L. ochrus* and *T. balansae* (Latham et al. 2001a). In an earlier study of CMV infection involving field surveys and replicated single row plots, yellow serradella became infected sporadically but developed no symptoms (McKirdy and Jones 1994b).

When exposed to AMV infection in single row plots, pink serradella proved highly susceptible and developed severe symptoms, but sulla did not become infected. *B. pelicinus* was susceptible and also developed very severe symptoms. Yellow serradella was moderately resistant and developed moderate symptoms. *T. balansae* was relatively resistant but infected plants developed severe symptoms. *L. cicera*, *L. sativus* and *L. ochrus* ranged from highly susceptible or moderately resistant and developed symptoms ranging from mild to severe. When inoculated in the glasshouse, sulla plants developed a localised hypersensitive reaction in inoculated leaves. AMV was seed-borne in *T. balansae* (7% seed transmission), *L. cicera* (2% seed transmission) and *L. sativus* (4% seed transmission) (Latham and Jones 2001a). In an earlier study of AMV infection involving field surveys and replicated single row plots, yellow serradella became infected at higher incidences than most other species. AMV was seed-borne in yellow serradella (0.1% seed transmission) (McKirdy and Jones 1994a).

These findings show that BYMV and AMV pose a potentially serious disease threat to *Biserrula* plantings, AMV poses a potential threat to pink and yellow serradella and BYMV to *T. balansae.* However, no information is available on their responses to challenge inoculation with other common pasture viruses such as CYVV, SCRLV, SCSV and WCMV.

Tedera is relatively insusceptible to AMV but occasionally became infected systemically developing a yellow mottle (Real D. and others, unpubl. data). Otherwise additional alternative pasture species of interest, e.g. lotus and tedera have not been assessed for their responses to virus infection.

No virus surveys of commercial plantings of these species have been undertaken and no virus-induced yield loss estimations have been made. Searches for virus resistance have not been made either, nor other studies on virus control measures.

9.12.3 What are the gaps and research needed?

The critical gaps that need addressing in relation to foliar diseases of other legumes for southern Australia are as follows:

- Survey pastures of alternative pasture legume species across southern Australia to establish relevant benchmarks for foliar fungal and viral pathogens and obtain yield loss data from grazed swards.
- Define relative resistances of current varieties and near-release breeding genotypes of alternative pasture legumes to the most important of the foliar fungal and viral pathogens.
- Define the strains of the most important viruses occurring in alternative pasture legume species across Australia such that available host resistances can be effectively deployed.
- For major fungal and viral diseases, define relative resistances of all near-release breeding genotypes across all alternative pasture species, and identify useful sources of resistances to these pathogens for breeders to utilize.
- Ensure seed stocks of new variety releases of alternative pasture species are free of seed-borne virus infection.

9.13 Virus infection in pasture legume breeding and selection programs

9.13.1 Contamination of seed stocks with seed-borne viruses

Pasture legume breeding and selection programmes are particularly vulnerable to contamination with seed-borne viruses transmitted non-persistently by aphids (Jones 1988, 1990, 2000, 2004a). When small plots are sown with diverse germplasm accessions originating in different countries alongside numerous genotypes or lines at different stages of breeding or selection, there is a high probability that seed-borne sources of virus infection will occur. If diverse legume species are being selected at the same site, this further increases the possibility that sources of more than one seed-borne virus are present. Also, there is often a concentration of numerous, small, widely-spaced, often single-row plots growing together and plots are often irrigated to prolong the growing period. These features encourage aphid activity thereby increasing the likelihood of virus spread within and between plots. At the end of the growing period, a high incidence of infection is associated with greater plot damage and virus infection in harvested seed (e.g., Jones 1988, 1990, 1999, 2000, 2004a; Jones and Pathipanawat 1989; Jones and McKirdy 1990). Unless control measures are taken, the risk of release of seed stocks of new varieties that are already virus contaminated is high resulting in contamination of farmers' pastures with seed- borne viruses. Measures are in place to avoid this when new subterranean clover varieties are released, especially as regards CMV seed contamination. However, such 'duty of care' precautions are not being taken with by lucerne and annual medic breeding programs resulting in widespread distribution of AMV- infected lucerne and annual medic seed to farmers and large-scale introduction of this virus to pastures. Other seed-borne viruses are also being introduced unknowingly in seed stocks of subterranean clover, lucerne and annual medic, and what introductions are occurring in seed stocks of other pasture legumes is unknown.

The principal factors favouring epidemics of AMV, BYMV and CMV in breeding, selection and seed multiplication plots of pasture legumes are those that: 1) increase the numbers of seed-infected plants (the primary virus source) and current-season infected plants (the secondary virus source), and the availability of both to aphid vectors; or 2) cause earlier arrival of aphid vectors, increase aphid landing rates and colonising aphid populations, and prolong aphid activity. The magnitude of the initial virus source depends on the amount of seed-borne infection and the extent of survival of seed-infected plants. Wide plant spacing's within rows and low seeding rates minimise competition between seed-infected and healthy seedlings, thereby favouring establishment of the former and prolonging their availability as sources for virus acquisition by incoming aphids before they become overshadowed by healthy growth. Similar considerations apply to early, secondarily-infected source plants as these become shaded over faster at close plant spacing's. If seed-borne viral infection is present in weeds in the seed bank at the site used and weed control is poor, germinating seed-infected weeds will further increase the primary virus infection source. Early arrival of aphid vectors in plots results from climatic conditions before sowing that favour rapid aphid build-up on surrounding vegetation, and, in consequence, early aphid flights. They also result from sowing plots just before annual peak aphid population times. Early aphid arrivals acquire the virus before seed-infected plants become overshadowed and initiate epidemics early. Wide plant spacing's within and between rows, and absence of non-host buffer crops and groundcover, prolong exposure of bare earth, attracting more incoming aphids to land and enhancing virus spread. Temperature and moisture conditions that favour build-up of colonising aphids within plots increase virus spread, especially by wingless aphids walking between intermingled pasture plants. Absence of insecticide applications has the same effect. Prolonged aphid activity is favoured by extended rainy seasons or irrigation, both of which increase the longevity of plants which results in greater final virus incidences and infection of harvested seed (Jones and Pathipanawat, 1989; Jones and McKirdy, 1990; Jones, 1999, Jones and Ferris, 2000; McKirdy et al., 2000; Latham and Jones, 2001a,b; Latham et al., 2001a; Jones 2004a).

9.13.2 Management of seed-borne viruses in pasture legume breeding, selection and seed increase programs

Because of the relatively small areas involved at pasture legume breeding and selection sites and seed- increase paddocks, and the high value of the germplasm, breeding lines and advanced selections being grown, use of control measures is invariably cost effective and there is scope for deploying additional measures that would otherwise be too expensive. A strategy was devised to control seed-borne CMV infection in subterranean clover breeding programmes. Jones and McKirdy (1990) briefly described the early phase of its development. It was later modified, improved and extended to control seed-borne AMV, CMV and BYMV in diverse species being bred or selected within general annual pasture legume improvement programmes (Jones 2004a).

Based on the earlier strategy for control of seed-borne CMV infection in subterranean clover breeding programmes and the critical epidemiological information described above on the factors that drive epidemics in plots of diverse pasture species, an Integrated Disease Management strategy was devised for AMV, BYMV and CMV at annual pasture legume improvement sites (Jones 2004a). Because of the great diversity of genetic material grown and the lack of effective biological control agents against aphid vectors, no host resistance or biological measures were possible. Only phytosanitary, cultural and chemical control measures are possible. The strategy is applied not only to small plots at early stages of selection but also to large plots during seed multiplication of selections identified for possible release (Jones 2004a). Although widespread adoption of the measures would improve the situation further, this approach has assisted in minimising the substantial damage to plots and contamination of seed experienced before control measures were first deployed, e.g. before 1988 within the subterranean clover breeding programme (Jones and McKirdy 1990).

AGWEST Plant Laboratories in WA provides a virus testing service for seed of pasture legume species.

9.13.3 What are the gaps and research needed for virus seed testing?

Research, development and application of new technologies to streamline virus seed testing services, such as quantitative real-time RT-PCR.

Provision of additional virus testing services for seed of pasture legume species by diagnostic laboratories.

Ensure that all pasture legume breeding selection and seed increase programs in Australia are made aware of their responsibilities not to distribute virus contaminated seed of new varieties and of the risks being taken when new pasture legume varieties are released without certification. This applies especially to the lucerne and annual medic programs.

Ensure that meat, dairy and wool growers understand (i) that a seed sample should have been tested for presence of seed-borne viruses before allowing seed onto their farms, and (ii) the risks from introducing seed-borne viruses being taken when new pasture legume varieties are introduced onto their farms.

9.14 Grasses

9.14.1 Soil-borne diseases of grasses

9.14.2 Disease symptoms and fungal and nematode pathogens involved, scope, extent and impact of soil-borne diseases on pasture productivity, and current management strategies

In 1987, Johnstone and Barbetti (1987) considered that research into the foliar diseases of pasture grasses in southern Australia had been totally neglected and that there was a general lack of information on distribution, incidence, and losses caused by them. Clarke and Eagling (1994), seven years later, reported that nothing had changed in this respect, in fact, if anything, the situation was now even worse for diseases of pasture grasses. Since then, little has changed. Of the little information available on grasses, it is largely only in the form of lists of possible pathogens (*e.g.*, Chambers 1959). Except for Clarke and Eagling (1994), there has been little or no attempt to even suggest which the most important root pathogens of grass species, let alone attempt to accurately define their impact or their relevance to rotational species. The limited interest in soil-borne grass diseases more often than not relates to the relevance of particular pathogens to cereals and the role of grass species in the disease epidemiology of cereal soil-borne diseases. For example, most pasture grasses are invariably susceptible to pathogens such as the ‘take all’ fungus (*Gauemannomyces gramminis* var. *tritici*) and *Rhizoctonia solani*. Grasses are known to be affected by root rot fungi and act as reservoirs providing carry-over inoculum for the following cereal crops (Cotterill and Sivasithamparam 1988b). Unfortunately, no effort has been made to date to evaluate the loss of productivity of the grass component of southern Australian pastures as a result of soil-borne pathogens causing root disease. To undertake this may be a particularly difficult undertaking, considering the variability not only in species composition of grasses, but also in the soil conditions that also affects the severity of root disease (Sivasithamparam 1993). Fungal pathogens have been demonstrated to have significant impact on productivity of ryegrass. Dewan and Sivasithamparam (1998a) showed that *Pythium* spp. in particular are highly pathogenic to ryegrass and can cause significant damage to roots, as were species of *Trichoderma* (1998b). *Penicillium griseofulvum*, *Aspergillus terreus*, *P. nigricans*, and *P. fuscum* isolated from ryegrass roots have also been shown to be pathogenic on ryegrass under some conditions (Dewan and Sivasithamparam 1998c). Glasshouse studies with cereal root pathogens have established the pathogenicity on ryegrass of the pathogen responsible for ‘take all’ disease, *G. graminis* var. *tritici*, and that it can cause significant damage to annual ryegrass (Dewan and Sivasithamparam 1998d). However, it is noteworthy that certain strains of *G. graminis* var. *tritici* which are highly pathogenic to wheat can indeed actually promote the growth of ryegrass (Dewan and Sivasithamparam 1990a,b). This was considered to be a significant finding at that time as it explained, for the first time, the dominance of ryegrass in ‘take all’ patches within wheat crops in the field in Western Australia. Both cocksfoot and tall fescue are also susceptible to fungal infections at the time of establishment (Clarke and Eagling 1994) but only evidence from outside Australia for this was provided in that report. Reports overseas showed that there is at least some potential for controlling grass soil-borne pathogens using fungicides, such as those involved in pre-emergence damping-off in both cocksfoot and tall fescue (Andrews 1953). An area where soil-borne pathogens have been observed to cause problems on grasses is during establishment (Clarke and Eagling 1994). It is likely that one or more species of *Pythium*, *Rhizoctonia* and *Fusarium* are associated with some establishment disorders in southern Australia (M.J. Barbetti, unpubl.). More recently, Simpson et al. (2011) used field-based plant bioassays to assess the potential for pre- and post-emergence loss of seedlings and for root damage affecting *Lolium rigidum x multiflorum* (annual ryegrass) seedlings during autumn-winter at several locations across a broad agricultural area of temperate southern Australia. Significant potential for loss of *Lolium rigidum x multiflorum* (annual ryegrass) seedlings was demonstrated at some of the sites.

The importance of root nematodes in Australian pasture grasses little studied. However, the role of grass weeds and grasses of self-regenerating pastures in the cropping zone in maintaining *Pratylenchus* populations, problematic pests of crops has been examined (Vanstone and Russ 2001). The data collected by Stirling and Lodge (2005) in two studies of contrasting areas in Australian, indicates that *Pratylenchus* is the most abundant endoparasitic nematode species and that some ectoparasites can be present in large numbers. These authors did not consider that the population densities found represented a significant problem. However, this finding needs be verified across a much wider range of agro-ecosystems.

Almost nothing is known in relation to potential host resistances to soil-borne pathogens that is available in grasses in Australia.

9.14.3 What are the gaps and research needed?

The critical gaps that need addressing in relation to soil-borne diseases of grasses for southern Australia are as follows:

- Survey grass pastures across southern Australia to establish relevant benchmarks for soil-borne disease incidence and impacts.

9.15 Foliar diseases of grasses

9.15.1 Disease symptoms and fungal foliar pathogens involved, scope, extent and impact of foliar diseases on pasture productivity, and current management strategies

Johnstone and Barbetti (1987) considered that research into the foliar diseases of pasture grasses in southern Australia had also been neglected and that there was a general lack of information on distribution, incidence, and losses caused by foliar pathogens. Again, Clarke and Eagling (1994), seven years later, reported that nothing had changed in this respect, and, as with soil-borne diseases, the situation remains little changed for diseases of pasture grasses. What little information is available on grasses is largely only in the form of lists of possible pathogens (*e.g.*, Chambers 1959). The grass components, especially in dry-land pastures used in rotation with crops in southern Australia, are what are considered by the cropping industries as predominantly weedy species, such as *Lolium*, *Bromus* and *Hordeum*. As a consequence, in Australia, most interest in diseases occurring in grasses relates to their relevance as pathogens or potential pathogens of cereals rather than as important pathogens of pasture pathogens in their own right. This is unfortunate as some diseases on pasture grasses clearly have major impact on their productivity (MJ Barbetti, unpubl.). For example, various rusts remain of relatively common occurrence on one or more of the main pasture grass species (MJ Barbetti, unpubl.), such as *Puccinia graminis lolii* on *Lolium* spp. and other grasses in Australia (Waterhouse 1951). Murray and Smith (1970) reported on a leaf blight disease of ryegrass (*Lolium* spp.) in New South Wales caused by *Cochliobolus sativus*. An isolated outbreak of this disease in 1968-69 killed grass and made the pasture unpalatable to stock and reduced production of the pasture by an estimated 75%. First symptoms were brown water-soaked spots, often with yellow halos. Heavily infected leaves became chlorotic and died. Other trash-/soil-borne pathogens commonly causing concern in grasses include *Cochliobolus sativus* (imperfect stage *Bipolaris sorokiniana*), *Drechslera* spp., and *Helininthosporium* spp. in perennial ryegrass (Clarke and Eagling 1994). Elsewhere, such as in the United Kingdom, Cook (1975) reported that other *Drechslera sissans* caused significant production losses in perennial ryegrass under high infection and nitrogen rates, and hence pathogens such as *Drechslera* spp. may also be much more important across southern Australia then currently realised.

Wong (1975) described kikuyu yellows, a disease that seriously reduces the productivity of affected kikuyu stands in NSW. The disease occurs as conspicuous yellow patches scattered randomly through established kikuyu pastures. Patches vary in diameter from 100 mm to more than 5 m and affected plants are stunted, unthrifty, and bear uniformly yellow leaves with characteristic brown flecking. Diseased stolons are easily pulled out of the ground and the roots are yellowish brown and partially rotted. Severely affected plants which are spindly with leaves crowded at the tips eventually die. Wong (1975) confirmed, using pathogenicity tests, that kikuyu yellows is caused by an undescribed oomycete fungus, which most resembles the genus *Achlya*. Allen *et al*. (1975) listed yellows as the most damaging disease of kikuyu. Rotation with crops requiring clean seedbeds and weed-free cultivation appears to reduce the incidence of the disease when a kikuyu pasture is re-established (Allen et al. 1975).

Almost nothing is known in relation to potential host resistances to foliar pathogens that are available to infect pasture grasses in Australia.

9.15.2 Disease symptoms and viral pathogens involved, scope, extent and impact of viral foliar diseases on pasture productivity, and current management strategies

Research into diseases of pasture grasses in southern Australia has been largely neglected, this being especially true before 1970 when diseases were not considered a significant problem. This perception has continued, even though a large body of evidence exists to show the detrimental effects of diseases in pasture grasses (Clarke and Eagling 1994). Diseases can cause reductions in establishment, dry matter production, palatability, feed quality, and seed production, and the insidious nature of some pathogens, particularly viruses, can hide the full extent of the problem and the amount of loss. Also, the development of improved grass pastures using introduced species has increased the likelihood of pastures suffering economic loss from introduced plant diseases, especially virus diseases (Clarke and Eagling 1994).

Virus diseases of Australian pasture grasses were reviewed briefly by Johnstone and Barbetti (1987), Panetta et al (1993), Clarke and Eagling (1994) and Jones (1996).

9.15.3 Disease symptoms – grass viruses

Important viruses

Barley yellow dwarf virus (BYDV) and *Cereal yellow dwarf virus* (CYDV): In the cooler climate of TAS, BYDV and CYDV normally cause symptomless infection in annual and perennial grasses. However, infected squirrel tail fescue (*Vulpia bromoides*) consistently showed leaf yellowing, perennial ryegrass (*Lolium perenne*) plants sometimes developed stunting, increased tillering and leaf yellowing, phalaris (*Phalaris aquatica)* plants occasionally developed stunting and leaf yellowing, and plants of tall fescue (*Festuca arundinacea*) and paspalum (*Paspalum dilatatum*) both occasionally developed yellowing of older leaves. Mixed infection of the same grass plant tended to increase symptom expression, but symptoms never developed in infected cocksfoot (*Dactylis glomerata*) plants (Guy et al. 1986, 1987; Guy
1988). In extensive over-summer surveys of grasses in WA which included most of these species, the important perennial pasture grass species kikuyu grass (*Pennisitum clandestinum*), and many others, apart from reddening of lower leaves of paspalum, neither BYDV or CYDV infection either alone or in combination were associated with plant obvious symptoms (Jones et al. 1990; McKirdy et al 1993; Hawkes and Jones 2005). In irrigated, mixed-species (white clover, perennial ryegrass and kikuyu grass) pastures sampled all-year-round in WA, no symptoms were associated with infection with BYDV or CYDV in either species (Coutts et al. 2002). In SA, no symptoms were reported in association with BYDV or CYDV infection in perennial grasses in irrigated pastures (Henry et al. 1992). In Victoria, there were no reports of BYDV symptoms in BYDV or CYDV infected perennial ryegrass in pastures (Eagling et al. 1989a,b, 1993; Clarke and Eagling 1984). Studies on BYDV/CYDV infecting samples of a range of pasture and weed grass species from QLD, VIC and NSW, did not report symptom expression in presence of infection either virus (Sward and Lister 1988).

Ryegrass mosaic virus (RyMV): RyMV causes an obvious light green to yellow streaky mosaic on leaves of perennial ryegrass and cocksfoot, or brown necrosis on leaves of Italian ryegrass (*Lolium multiflorum*) (Slykhuis and Paliwal 1972; Eagling et al. 1992).

Wheat streak mosaic virus (WSMV): WSMV infects a range of annual and perennial wild grasses in Australia. Infection of African lovegrass (*Eragrostis curvula*), whorled pigeon grass (*Setaria verticillata)*, spike goose-grass (*Eleusine tristachya*) and a panic (*Panicum* sp.) was associated with leaf streaking and mottling in the ACT (Ellis et al. 2004), but no symptoms were associated with natural infection of a wide range of annual grass species in WA (Coutts et al. 2008a,b). WSMV symptoms have not been studied in the major grass species sown in pastures.

Other grass viruses

In VIC and TAS (see Guy and Sward 1991 for more information): *Ryegrass cryptic virus.*

In grasses in QLD (see Buchen-Osmond et al. 1988 for more information): *Cereal chlorotic mottle rhabdovirus, Chloris striate mosaic virus, Digiataria striate mosaic virus, Johnson grass* *mosaic virus, Maize mosaic rhabdovirus, Maize sterile stunt rhabdovirus, Maize stripe tenuivirus, Pangola (Australian) stunt fiji virus, Paspalum striate mosaic virus, Sugar cane fijivirus, Sugar cane mosaic virus.*

9.15.4 Extent and impact of viral diseases on pasture productivity

BYDV/CYDV: BYDV and CYDV infect perennial ryegrass in VIC, SA, NSW, TAS, QLD and WA and infection occurs in grasses in perennial pastures throughout south-eastern and south-western Australia (Smith 1964; Guy et al. 1987; Greber 1988; Eagling et al. 1989; Jones et al. 1990; Henry et al. 1992; McKirdy et al. 1993; Coutts and Jones 2002).

Guy et al. (1986) tested samples of perennial ryegrass, cocksfoot, tall fescue, phalaris and timothy (*Phleum pratense*) from 29 pastures in TAS two thirds of which were no more the 2 years old, and detected BYDV/CYDV in 13% of the samples. Only tall fescue and perennial ryegrass were infected, and the highest incidence of infection was 70% in a four-year-old perennial ryegrass pasture. They predicted that testing of older samples would have revealed higher infection levels. In 1983-1985, Guy (1988) tested grass samples from two sets of plots of perennial ryegrass, phalaris, tall fescue and cocksfoot sown in 1983 in TAS. The samples were collected at regular intervals over 2.5 years, and within infected samples the viruses incidences were BYDV (74%), CYDV (26%) and both together (17%). Virus incidence in tall fescue (21%) and perennial ryegrass (27%) was significantly greater than in cocksfoot (6%) or phalaris (7%). Mixed infection and BYDV were more common than CYDV in tall fescue and perennial ryegrass, but the incidence of CYDV was greater in cocksfoot and phalaris.

Sward and Lister (1988) reported that 86% and 21% of grass samples tested from NSW and VIC in 1985-1986, respectively, were infected with BYDV/CYDV. These samples were mixtures of several different grass species including perennial ryegrass, Italian ryegrass, paspalum, cocksfoot and several others. In VIC, when the incidence of BYDV in perennial ryegrass swards growing at three sites was investigated in 1987-1988, BYDV/CYDV infection was frequent but varied with variety and site. Infection incidences were up to 95% in cv. Victorian and up to 80% in cv. Tasmanian No. 1, but the highest incidence in cv. Ellet was only 34% (Eagling et al. 1989a). In SA, when the incidence of BYDV in perennial grass pastures containing mostly perennial ryegrass, cocksfoot, paspalum, tall fescue and brome grass (*Bromus catharticus)* was examined at 24 sites in 1990-1991, infection with BYDV/CYDV was always present but its overall incidence in combined grass species samples varied with site and year reaching a maximum of 86% Perennial ryegrass constituted 52% of the total sample number, and the overall percentages of BYDV/CYDV infection in samples of different species regardless of site or year was 30% (perennial ryegrass), 56% (tall fescue), 9% (cocksfoot), 9% (brome grass) and 2% (paspalum) (Henry et al. 1992).

In an over-summer survey in WA in 1989, Jones et al. (1990) detected BYDV and/or CYDV in grass samples from 72 sites. BYDV or CYDV were detected at 71% and 86% of sites and both at 57% of sites, often as mixed infections within the same plant. They detected BYDV and CYDV in Kikuyu grass, paspalum and several weed grasses, and BYDV in ryegrass (perennial or annual). McKirdy and Jones (1983) undertook a further over-summer survey in WA in 1991-1992 which included irrigated pastures and other locations where grasses were growing wild. They detected BYDV/CYDV in Kikuyu grass (57% of samples, 58% of sites), paspalum (21% of samples, 25% of sites), and in many non-pasture, annual and perennial grass species. In an over-summer survey in 2001-2002, Hawkes and

Jones (2005) sampled wild grasses in the WA grainbelt. The principle grasses also sown in pastures that they sampled were Kikuyu grass (7-8% BYDV/CYDV infection) and Rhodes grass (*Chloris virgata*) (2-3% BYDV/CYDV infection). Greatest infection incidences at individual sites were 23% CYDV in Kikuyu grass and 26% BYDV in Rhodes grass both in 2000 (Coutts and Jones 2002). When Coutts and Jones (2002) sampled six mixed species, irrigated perennial pastures in WA at regular intervals over 10-22 months, BYDV incidence in perennial ryegrass fluctuated but never exceeded 17%, while its incidence in Kikuyu grass never exceeded 4%.

BYDV infection results in reductions in grass pasture establishment, competitiveness, persistence, productivity (Catherall and Parry 1987), and quality (Eagling and Sward 1989b), the extent of changes in plant growth and metabolism depending on the prevailing environmental conditions. In field experiments with perennial ryegrass in the UK, BYDV reduced dry matter yield by up to 24%, with reductions of 17% and 8% in annual herbage from swards cut twice or four times a year (Catherall 1996; Wilkins and Catherall 1997). Also, infection reduced its competitive ability resulting in poorer stand establishment (Catherall 1987; Catherall and Parry 1987). Coutts and Jones (2002) did not find a clear relationship between incidence of BYDV infection of perennial ryegrass and the relative species balance within grazed and mown mixed grass-white clover pastures in WA, but BYDV-infected Kikuyu grass (*P. clandestinum*) plants seemed less able than healthy Kikuyu grass plants to survive competition with white clover and ryegrass plants. No other research in Australia has examined effects of BYDV infection on productivity of grass swards. However, in a spaced plant experiment in VIC with perennial ryegrass plants and BYDV), dry weights were significantly greater in cv. Ellet genotypes classed as BYDV resistant than as susceptible (dry weight reduction from BYDV infection of up to 22%), but there was no significant difference in dry weights between these genotypes in the absence of BYDV (Eagling et al. 1993. Also, Eagling et al. (1989b) used controlled environment rooms to measure effects of BYDV infection on early growth of commercial varieties of perennial ryegrass (*Lolium perenne*) (1 variety, Victorian), Italian ryegrass (*Lolium multiflorum*) (2 varieties, Grasslands Tama and Barvestra) and a hybrid variety between the two species (cv. Grasslands Ariki) under two different temperature regimes (24°C and 16°C). At 24°C, BYDV infection was associated with reduced root dry weight (30-40%) in all five varieties, but the effect of infection on shoot dry weight and leaf area was variable. At 16°C, the effect of BYDV infection was variable, being associated with increases in root dry weight, shoot dry weight, and leaf area in one variety (Grasslands Ariki) and decreases in another (Victorian). In two other varieties, root dry weight, shoot dry weight and leaf area were not significantly affected. At 24°C, reductions in root dry weight associated with infection were not concomitant with reductions in the root relative growth rates. Up to at least 46-50 days after germination reductions in root dry weight were associated with both aphid-feeding damage and virus infection. Experiments with cv. Victorian, showed that shoot dry weight was not significantly affected by feeding with viruliferous or non-viruliferous oat aphids (*Rhopalosiphum padi*). At 16°C, changes in root and shoot dry weight were associated with changes in the root and shoot relative growth rates (Eagling et al. 1989b).

Research on BYDV in grasses in Australia has also revealed the roles that perennial ryegrass, Kikuyu grass, tall fescue, paspalum and other pasture grass species all play as sources of BYDV for infection of nearby cereal crops and in allowing survival of aphid vectors of BYDV during the cool winter period in VIC or the dry summer period in WA and SA (Clarke and Eagling 1994; Henry et al. 1992; McKirdy and Jones 1993; Hawkes and Jones 2005).

RyMV: Although RyMV infection occurs in perennial grass pastures in TAS, VIC and WA, no large-scale surveys for RyMV infection in pastures have been reported outside WA. Sampling of grasses in perennial pastures in high rainfall districts of VIC indicated that RyMV was present (Eagling et al. 1992), but no incidence data were given. Similarly, although RyMV is reported from grass pastures in TAS (Guy and Sward 1991), incidence data are lacking. In WA, McKirdy and Jones (1997) detected up to 34% incidences of RyMV in the perennial ryegrass component of 11/18 irrigated, mixed white clover and grass pastures. All these pastures were <6 years old and their ryegrass components were persisting poorly, apparently due to inability of RyMV-infected ryegrass plants to compete with white clover, weeds and other grass species. This inability was not due to BYDV infection of ryegrass in these pastures as its incidence never exceeded 4% in them. When Coutts and Jones (2002) sampled six mixed species, irrigated perennial pastures in WA at regular intervals over 10-22 months, RyMV incidences in perennial ryegrass fluctuated but were generally high, reaching 73% infection. Such incidences are likely to reduce the competitive ability of ryegrass resulting in predominance of other pasture species and weeds. The virus was absent from Kikuyu grass also growing in these pastures but an unidentified potyvirus was sometimes present in Kikuyu grass in addition to BYDV.

In the U.K., RyMV infection causes reductions in herbage of up to 26% in perennial ryegrass (A'Brook and Heard 1975) and 20% in Italian ryegrass (Jones et al. 1977) as well as reductions in response to nitrogen applications (A'Brook and Heard 1975). In VIC, RyMV infection was studied over 2 years in simulated swards made up of spaced plants of Italian ryegrass cv. Grasslands Tama and perennial ryegrass cvs Ellet and Wimmera. In early tests, the highest incidence of RyMV infection was 47% in cv. Grasslands Tama, while lower levels of infection (24-15%) were found in cvs Ellet and Wimmera. However, in the cv. Ellett plants that had survived into the second year, there was a 46-88% RyMV incidence. When sward composition was determined, the ryegrass component of the RyMV-infected plots was reduced by up to 50%, the extent of these reductions due to RyMV infection varying with ryegrass variety. These reductions in ryegrass content were associated with compensatory weed growth showing that infection with RyMV reduced the competitive ability of the infected ryegrass (Eagling et al. 1992). In TAS, *Ryegrass cryptic virus* (Guy and Sward 1991), which has no direct effect on ryegrass species, enhances the severity of RyMV infection when present in mixed infections (Clarke and Eagling 1994).

WSMV: Ellis et al (2003) detected WSMV in four grass species growing in the ACT but infection incidences were not reported. Surveys of the occurrence of WSMV in grasses in the grainbelt region of WA found this virus infecting a range of annual grass weeds growing as wild plants. Annual ryegrass (*Lolium rigidum*), barley grass (*Hordeum sp*.), wild oats (*Avena fatua*), small burr grass (*Tragus australianus*), stink grass (*Eragrostis cilianensis*), and witch grass (*Panicum capillare*) were infected. Incidences of infection at individual sites reached 65% in annual ryegrass (Coutts et al. 2009a,b). Surveys are needed to establish how widespread infection with WSMV has become in grasses growing within pastures in VIC, TAS, SA, WA, QLD and NSW as the virus now occurs commonly in these states.

No yield loss determinations have been made to determine the effect of WSMV on productivity of infected pasture grasses. However, research on WSMV in grasses in Australia has revealed the important roles that infected grasses play as sources of WSMV for infection of nearby cereal crops and in allowing survival of wheat curl mite (WCM) vectors of WSMV during the dry summer period (Coutts et al. 2006b).

9.15.5 Aetiology and epidemiology – virus diseases

BYDV/CYDV: BYDV was first reported in Australia by Smith (1957), Geard (1960) and Slykius (1962). It occurs in VIC, TAS, SA, WA, QLD and NSW (Buchen-Osmond et al. 1988). Australian isolates of BYDV formerly consisted of four strains, PAV, MAV, RMV and RPV, and detailed surveys established the incidences of all four strains in pasture and wild grasses in TAS, VIC, SA and WA Subsequently, the International Committee on the Taxonomy of Viruses (ITCV) divided the BYDVs into two viruses, BYDV (PAV, RMV and MAV) and CYDV (RPV) (D'Arcy et al., 1999).

BYDV/CYDV infects more than 100 species of Poacae, including Australian pasture grasses, such as perennial ryegrass, cocksfoot, tall fescue, phalaris, timothy, Kikuyu and Rhodes grass (Guy et al. 1987; Greber 1988; Eagling et al. 1989; Clarke and Eagling 1994; Jones et al. 1990; Henry et al. 1992; McKirdy et al. 1993; Coutts and Jones 200; Hawkes and Jones 2005). Both viruses are transmitted persistently by aphids but are not seed-borne. Their principal vectors in Australian pasture grasses are the oat aphid (*Rhopalosiphum padi*) and the corn leaf or maize aphid (*R. maidis*) (Guy 1988; McKirdy et al. 1993; Coutts and Jones 2002). However, other BVYDV/CYDV aphid vectors sometimes also occur on pasture grasses, including the grain aphid (*Sitobion miscanthi*), the blackberry cereal aphid (*S. fragariae*), the rose-grain aphid (*Metapolophium dirhodum*) and (Guy et al. 1987). The rose-grain aphid only arrived in Australia in 1984 (Carver 1984; Waterhouse and Helms 1985) and has not yet reached WA. Infestations with the rusty plum aphid (*Hysteroneura setariae*) and mealy plum aphid (*Hyalopterus pruni*) are common in pasture and wild grasses in WA and TAS respectively but neither species is recorded as a vector of BYDV or CYDV (Guy et al. 1987; McKirdy and Jones 1993; Coutts and Jones 2002).

The epidemiology of BYDV/CYDV in perennial grass pastures is influenced by the strains involved, aphid vectors present in the area, pasture management practices, environmental conditions (temperature and rainfall) and the timing of aphid flights. The classic relationship between BYDV stains and vector aphid species is: PAV is transmitted regularly by *R. padi* and *Sitobion* spp., but rarely by *R. maidis*; MAV is transmitted regularly by *Sitobion* spp., but rarely by *R. padi or R. maidis;* and RMV is transmitted regularly by *R. maidis*, but infrequently by *R. padi or Sitobion spp.* CYDV (=RPV) is transmitted regularly by *R. padi*, but rarely by *R. maidis* or *Sitobion spp.* (Rochow 1970). Within established perennial pastures aphids acquire BYDV or CYDV from infected grass plants within the pasture sward and transmit it to healthy plants. Transmission to adjacent intermingling plants is mostly by wingless aphids but creation of new infection foci distant from the primary virus focus is by winged viruliferous aphids. Moreover, once BYDV or CYDV achieve high incidences these are unlikely to decline in subsequent years because once a plant becomes infected it remains infected for the rest of its life. Even if the tops die off and the roots remain during the hot, dry summer period, their roots are still infected and infected plants will grow back from them. In contrast, with annual grasses the annual epidemic has to start off again from a low base every year. As mentioned above, BYDV-infected Kikuyu grass plants in mixed species pasture seem less able than healthy Kikuyu grass plants to survive competition with healthy white clover or perennial ryegrass plants (Coutts and Jones 2002). Such effects of BYDV infection can result in alteration of the species balance within pasture swards such that the proportion of the infected grass species declines in relation to other pasture species or weeds.

RyMV: RyMV was first diagnosed in Australia in ryegrass cv. Grasslands Tama in Tasmania in 1986 (Guy and Sward 1991). Its cereal rust mite vector *(Abacarus hystrix*) was first recorded in TAS in 1989 (Frost et al. 1990) and is widely distributed through the higher rainfall (>700 mm) districts (and elsewhere in irrigated perennial pastures) in SA, VIC, and southern NSW (Clark and Eagling 1994). The virus occurs naturally in Italian ryegrass (*L. multiflorum*), perennial ryegrass (*L. perenne*) and cocksfoot (*Dactylis glomerata*). The mite vector acquires the virus when it feeds on infected ryegrass plants and spreads it to healthy plants when it moves onto them and feeds. The intensity and frequency of grazing have major influences on rust mite populations and hence on spread of RyMV. Pastures which are grazed heavily and infrequently generally have lower rust mite populations than those with light, frequent grazing schedules (Frost 1993). The influence heavy grazing schedules have on RyMV spread is likely to be enhanced in summer, when removal of herbage by grazing can expose remaining mites located at the bases of plants in short swards to potentially lethal temperatures they would not otherwise experience (Clarke and Eagling 1994). As mentioned above, in WA RyMV-infected perennial ryegrass plants in mixed species pasture were less able than healthy ryegrass plants to survive competition with healthy white clover, Kikuyu grass or weeds (McKirdy and Jones 1997; Coutts et al. 2002). In VIC, such reductions in ryegrass content in monoculture swards were associated with compensatory weed growth (Eagling et al. 1992).

WSMV: The first definitive report of WSMV in Australia was 2003 in wheat breeding facilities at Canberra in the ACT where the virus was found in grass species in addition to wheat (Ellis et al. 2003). Soon afterwards, it was found at diverse sites in SA, NSW, QLD and VIC. In WA, WSMV was not found until 2006, when it was detected in wheat and several grasses (Coutts et al. 2009a,b). Subsequent studies with isolates from different parts of Australia and elsewhere suggested that WSMV probably arrived 10– 20 years ago, as a result of a single introduction of infected wheat seed containing a WSMV genetic variant originally from the Pacific North West of the USA (Dwyer *et al*. 2007)

WSMV is transmitted from infected to healthy grass plants by the wheat curl mite (WCM, *Aceria tosichella*). WCM is tiny and wingless and only crawls short distances. Unless plants are touching, it requires wind to move it from plant to plant. It also requires movement by wind to initiate new infection foci distant from the infection source. Although WCM is carried passively on air currents, active initiation of movement occurs, the mites moving to the leaf edge, leaf tip, or awn and standing upright facing the wind before take-off. Temperature and rainfall in summer and early autumn are key factors influencing WCM population build up in Australia. The optimal temperature for rapid mite increase, population development and movement is 24-27^{o}C so warm summer conditions and abundant grass growth provide ideal circumstances for rapid increases in WCM populations and WSMV spread (see Coutts et al. 2009b). Another key factor influencing WCMV epidemics is availability of WSMV inoculum. Seed- infection provides a potential source through sowing infected seed. However, although we know WSMV is seed-borne in wheat (Jones et al. 2005), we do not know yet if it is seed-borne in any grasses. Should this be so, sowing infected seed would not only introduce the virus to grasses in pastures but also provide internal sources within pastures for WSMV acquisition and spread by WCM. Since infection with WSMV can be common in wild grasses and annual grasses within pastures in WA and ACT (Ellis et al. 2003; Coutts and Jones 2009a,b), infection is also likely to be common in grasses in perennial grasses within pastures but this remains to be demonstrated. As mentioned above, the grass hosts identified so far in Australia are African lovegrass, whorled pigeon grass, spike goose-grass, a panic, annual ryegrass, barley grass, wild oats, small burr grass, stink grass, and witch grass (Ellis et al. 2003; Coutts and Jones 2009a,b).

9.15.6 Current management strategies – virus diseases

BYDV/CYDV: There is a need to ensure adequate sources of host resistance are available to introduce into ryegrass breeding programmes to combat CYDV and the three strains of BYDV in Australia. This will ensure that varieties developed from elite germplasm do not have enhanced susceptibility to either virus, resulting in their failure in the field when commercially released (Clarke and Eagling 1984). In south- eastern Australia, BYDV-PAV predominates in infected perennial ryegrass pastures (Guy et al. 1986; Eagling et al. 1989), and accordingly, breeding programmes in VIC have focussed on selecting resistance to this isolate. However, in TAS, selection programmes have also included resistance to CYDV and BYDV-MAV, with the CYDV being more than BYDV common on cocksfoot and phalaris (Clarke and Eagling 1994).

In VIC, BYDV infection is less frequent in perennial ryegrass cv. Ellett than cv. Victorian (Eagling et al. 1989b). Different genotypes of cv. Ellett were classed as susceptible or resistant to infection with BYDV- PAV on the basis of dry weight yield and the presence or absence of detectable virus. In addition, superior plants were selected from four varieties of perennial ryegrass on the basis of superior winter growth and seasonal production. In glasshouse experiments, resistance to the virus was detected in these selections. The percentage of infected selections from individual varieties varied between 18-46%, indicating that at least 50% were resistant (Eagling et al. 1993). Thus, sources of BYDV resistance are available in Australia that can be used to breed BYDV-resistant pasture ryegrass.

BYDV/CYDV can be controlled effectively and cheaply in Australian cereal crops by removal of grass weeds by applying herbicide before sowing, manipulation of sowing date, carefully timed application of pyrethroid insecticide, insecticidal seed dressings and planting varieties with virus resistance (e.g. McKirdy et al. 1996, 1997). However, no research has been undertaken in Australia to explore the effectiveness of manipulation of grazing or species composition, cultural methods, or chemical methods against aphid vectors to control BYDV/CYDV spread in perennial grass pastures.

RyMV: There is a needed to ensure adequate sources of host resistance are available to introduce into Australian ryegrass breeding programmes to combat different strains of RyMV. This will ensure that varieties developed from elite germplasm do not have enhanced susceptibility to this virus, resulting in failure when commercially released (Clarke and Eagling 1984). In the UK, resistant plants of perennial ryegrass (Gibson and Heard 1979) possess two independently inherited types of resistance (Sulehuzzamen and Wilkins 1984). Also, success in transferring polygenic resistance to RyMV from perennial ryegrass to varieties of Italian ryegrass (Wilkins 1987) suggested the possibility of developing Italian ryegrass varieties resistant to RyMV. Resistance to RyMV occurs in individual selections of perennial ryegrass that can be used to breed for resistance to RyMV in Australia (Clarke and Eagling 1994). No research has been undertaken in Australia to explore the effectiveness of manipulation of grazing or species composition, cultural methods, or chemical methods against mite vectors to control RyMV spread in pastures.

WSMV: No studies on control measures have been investigated for suppressing spread of WSMV in grass pastures. There is scope for exploring the effectiveness of host resistance, manipulation of grazing or species composition, cultural methods and chemical methods against WCM to control its spread in pastures.

Other viruses: There is a need to keep a watching brief on ryegrass and other pasture species for grass viruses isolated in QLD that have not yet been detected elsewhere in Australia, or viruses that are not as yet known to be present in Australia. For example, there are a number of viruses of cocksfoot not as yet been detected in Australia, the more economically significant of these being cocksfoot mottle, cocksfoot mild mosaic and cocksfoot streak viruses (Clarke and Eagling 1984). These viruses have been the subject of several screening projects and promising sources off resistance have been found, including resistance to cocksfoot mottle virus in cv. Grasslands Nui which is also resistant to cynosurus mottle mosaic virus (Braverman 1986). Also, Braverman (1986) reported that phalaris is resistant to a range of viruses including maize dwarf mosaic, maize chlorotic dwarf, sugar cane mosaic, and cocksfoot mottle.

9.15.7 What are the gaps and research needed?

The critical gaps that need addressing in relation to foliar diseases of grasses for southern Australia are as follows:

- Survey grass pastures across southern Australia to establish relevant benchmarks for important foliar disease fungal and viral pathogen impacts and obtain yield loss data from grazed swards.

- Define relative resistances of current varieties and near-release breeding genotypes of grasses to the most important of the foliar fungal and viral pathogens.

- Define the strains of the most important viruses (BYDV, CYDV, RyMV) occurring in grass pasture species across Australia such that available host resistances can be effectively deployed.

- Determine the occurrence and importance of newly introduced WSMV in Australian grass pastures.

- For major fungal and viral diseases, define relative resistances of all near-release breeding genotypes across grass pasture species and identify useful sources of resistances to these pathogens for breeders to utilize.

9.16 References

Abbo, E.O., Irwin, J.A.G. 1990. *Aphanomyces euteiches*, a possible cause of poor Lucerne establishment in the Lockyer Valley, Queensland. *Australian Journal of Experimental Agriculture* 30, 361-364.

A'Brook, J., Heard, A.J. 1975. The effect of ryegrass mosaic virus on the yield of perennial ryegrass swards. *Annals of Applied Biology* 80, 163-8.

Aftab, M., Freeman, A. 2006. Temperate pulse viruses: cucumber mosaic virus (CMV). Information Note No. AG1207, Victorian Department of Primary industries, Horsham.

Aitken, Y, Grieve, B.J. 1943. A mosaic disease of subterranean clover. *Journal of the Australian Institute of Agricultural Science* 8, 81-82.

Alberts E (1995) Alfalfa mosaic virus in lucerne and medic seed and development of rapid tests. Final Report for the Rural Industries Research and Development Corporation, September 1995. 22p. (SARDI: Adelaide, S. A.)

Allen, R.N., Wong, P. and Walker, J. 1975. Diseases and pests of kikuyu grass. *Agricultural Gazette, New South Wales* 86, 18.

Anderson, W.K., Parkin, R.J. and Dovey, M.D. 1982. Relations between stocking rate, environment and scorch disease on grazed subterranean clover pasture in Western Australia. *Australian Journal of Experimental Agriculture and Animal Husbandry* 22, 182-189.

Andrew, W.D. 1963. Susceptibility to damping-off in seedlings of *Medicago denticulata* Willd. and *M. minima* (L.) Bart. during the early post-emergence period. *Australian Journal of Biological Sciences* 16, 281-3.

Andrews, E.A. 1953. Seedling blights and root rot of forage grasses. *Disease*

Abstracts 13, 692. Anon. 1960. Victorian Department of Agriculture, Report of the

Biology Branch 1. Anon. 1964. Diseases of clovers in New South Wales.

Agricultural Gazette of New South Wales 75,1204-11.

Anon. 1965. Annual Report, NSW Department of Agriculture, p. 76.

Anon. 1966. Clover rust. *Agricultural Gazette, New South Wales* 77, 257 and cover.

Anon. 1968. Annual Report, NSW Department of Agriculture, p. 99.

Anon. 1968. Red leaf - a new virus disease that can make subterranean clover totally unproductive. *Journal of the Department of Agriculture of Victoria* 66, 182 - 184.

Anon. 1969. Annual Report, NSW Department of Agriculture, p. 159.

Anon. 1970. Annual Report, NSW Department of Agriculture, p. 201.

Anon. 1971. Annual Report, NSW Department of Agriculture, pp. 29 and 33.

Anon. 1972. Diseases of lucerne. Department of Agriculture, New South Wales, Plant Disease Bulletin 58.

Anon. 1975a. Clover diseases in New South Wales. Department of Agriculture, New South Wales, Bulletin 155.

Anon. 1975b. Root rot of lucerne. *Agricultural Gazette, New South Wales* 86, 48.

Anon. 1988. The economic impact of pasture weeds, pests and diseases on the Australian wool industry: main report. Report by Sloane, Cook and King Pty Ltd. for the Australian Wool Corporation.

Ballard, R.A., Hutton, R.E., Taylor, S.P., McKay, A.C., Howie, J.H. 2006. Field resistance of annual pasture legumes to the root lesion nematode, *Pratylenchus neglectus*. *Australasian Plant Pathology* 35, (in press).

Barbetti, M.J. 1983a. Fungicidal control of damping-off and seedling root rot in subterranean clover. *Fungicide and Nematicide Tests* 38, 47.

Barbetti, M.J. 1983b. Diseases and their control in lucerne. Department of Agriculture, Western Australia, Farmnote 94/83.

Barbetti, M.J. 1983c. Fungicidal control of damping-off and seedling root rot in subterranean clover. *Fungicide and Nematicide Tests* 38, 47.

Barbetti MJ 1983d. Fungal foliage diseases of pasture legumes. *Journal of Agriculture, Western Australia* 1, 10-12.

Barbetti, M.J. 1983e. Cercospora disease on pasture legumes. Department of Agriculture, Western Australia, Plant Research Division Annual Report 1982/83 pp. 124-6.

Barbetti, M.J. 1983f. *Cercospora zebrina* on pasture legumes in Western Australia. 4th International PlantPathology Congress, Melbourne, August 1983. Abstract of Papers p. 222 (Abstract No. 886).

Barbetti, M.J. 1984a. Seed treatments for control of subterranean clover root rot. *Australasian Plant Pathology* 13, 43-45.

Barbetti, M.J. 1984b. Ridomil and Benlate soil drenches for control of damping-off and seedling rot in subterranean clover. *Fungicide and Nematicide Tests* 40, 120.

Barbetti, M.J. 1984c. Subterranean clover foliage fungi as root pathogens. *Australasian Plant Pathology* 13, 38-40.

Barbetti, M.J. 1984d. Relation between plant size and severity of root rot in subterranean clover. *Australian Journal of Experimental Agriculture and Animal Husbandry* 24, 126-129.

Barbetti, M.J. 1985a. Apron seed treatments for control of damping-off and seedling root rot in subterranean clover. *Fungicide and Nematicide Tests* 41, 128-129.

Barbetti, M.J. 1985b. Infection studies with *Cercospora zebrina* on pasture legumes in Western Australia. *Australian Journal of Experimental Agriculture* 25, 850-855.

Barbetti, M.J. 1985c. Inter-relationships between *Cercospora zebrina* and *Kabatiella caulivora* on subterranean clover. *Australasian Plant Pathology* 14, 1-2.

Barbetti, M.J. 1985d. Survey of fungi associated with subterranean clover leaves and petioles in Western Australia. *Plant Pathology* 34, 49-53.

Barbetti, M.J. 1986. Effect of season, trash and fungicides on fungi associated with subterranean clover. Australian *Journal of Experimental Agriculture* 26, 431-435.

Barbetti, M.J. 1987a. Effects of three foliar diseases on biomass and seed yield for 11 cultivars of subterranean clover. *Plant Disease* 71, 350-353.

Barbetti, M.J. 1987b. Evaluation of fungicides for control of *Cercospora zebrina* on subterranean

Barbetti, M.J. 1987c. Effects of temperature and humidity on disease caused by *Phoma medicaginis*, resistance in some *Medicago* cultivars and the incidence of seed-borne inoculum. *Australian Journal of Experimental Agriculture* 27, 851-856.

Barbetti, M.J. 1987d. Seasonal fluctuations in concentration of air-borne conidia of *Cercospora zebrina* and incidence of Cercospora blackstem disease in subterranean clover. *Transactions of the British Mycological Society* 88, 280-283.

Barbetti, M.J. 1989a. Strategies for control of Phoma black stem in annual *Medicago* species. *Australian Journal of Experimental Agriculture* 29, 635-460.

Barbetti, M.J. 1989b. Fungal diseases of pasture legumes in Western Australia. Department of Agriculture, Western Australia, Bulletin No. 4133, pp.21.

Barbetti, M.J. 1989c. Response of *Medicago* cultivars to fungal root pathogens associated with *Trifolium subterraneum*. *Plant Protection Quarterly* 4, 1-3.

Barbetti, M.J. 1989d. Response of subterranean clover cultivars to *Phytophthora clandestina*. *Phytophylactica* 21, 65-67.

Barbetti, M.J. 1990a. Effect of adding lime to potting soil upon the pathogenicity of four root pathogens on subterranean clover. *Phytophylactica* 22, 245-249.

Barbetti, M.J. 1990b. Resistance in annual *Medicago* species to *Phoma medicaginis* under controlled environment and field conditions. *Australian Journal of Experimental Agriculture* 30, 209-214.

Barbetti, M.J. 1990c. Fungi associated with seed of subterranean clover (*Trifolium subterraneum*) in Western Australia. *Seed Science and Technology* 18, 673-679.

Barbetti, M.J. 1991a. Effect of clover-free rotations upon the severity of root rot and yield in regenerating subterranean clover pastures. *Australian Journal of Agricultural Research* 42, 1195-1204.

Barbetti, M.J. 1991b. Response of subterranean clover varieties to Cercospora disease under a controlled environment and in the field. *Australian Journal of Experimental Agriculture* 31, 333-336.

Barbetti, M.J. 1992. Efficacy of fungicides for control of Phoma black stem and Pseudopeziza leaf spot in annual *Medicago polymorpha* var. *brevispina*. *Plant Protection Quarterly* 7, 21-22.

Barbetti, M.J. 1993a. Phoma black stem disease – a threat to susceptible annual medic pastures. In: Proceedings of the XVII International Grasslands Congress, Palmerston North, New Zealand, pp. 929-930.

Barbetti, M.J. 1993b. Clover scorch and its control. Department of Agriculture Western Australia. Farmnote 76/93.

Barbetti, M.J. 1995a. Relative resistance, associated yield losses and phyto-oestrogen production from fungal foliar diseases in new and old annual *Medicago* cultivars. *Australian Journal of Agricultural Research* 46, 441-450.

Barbetti, M.J. 1995b. Resistance in annual *Medicago* species to *Phoma medicaginis* and *Leptospherulina trifolii* under field conditions. *Australian Journal of Experimental Agriculture* 35, 209-214.

Barbetti, M.J. 1995c. Breakdown in resistance of subterranean clovers to clover scorch disease (*Kabatiella caulivora*). *Australian Journal of Agricultural Research* 46, 645-653.

Barbetti, M.J. 1996. The successful control of clover scorch disease (*Kabatiella caulivora*) in Australian subterranean clover pastures. *In*: Pasture and Forage Crop Pathology. Pages 589-601. Eds Chakraborty, S., Leath, K.T.L., Skipp, R.A., Pederson, G.A., Bray, R.A., Latch, G.C.M. and Nutter, F.W. ASA, CSSA, and SSSA, Madison, WI.

Barbetti, M.J. 2005. *Cylindrocarpon didymum* - a root pathogen of subterranean clover in the lower south west of Western Australia. *Australasian Plant Pathology* 34, 111-114.

Barbetti, M.J. 2007a. Resistance in annual *Medicago* spp. to *Phoma medicaginis* and *Leptosphaerulina trifolii* and its relationship to induced production of a phyto-oestrogen. *Plant Disease* 91, 239-244.

Barbetti, M.J. 2007b. The expression of resistance in subterranean clover (*Trifolium subterraneum*) to races 1 and 2 of *Kabatiella caulivora* is affected by inoculum pressure but not by combinations of the two races. *Australasian Plant Pathology* 36, 318-324.

Barbetti, M.J. and Allen, J.G. 2005. Association of *Fusarium* species, with potential for mycotoxicosis, on pods of annual *Medicago* in Western Australia. *Australian Journal of Agricultural Research* 56, 279-284.

Barbetti, M.J. and Allen, J.G. 2007. Toxigenic *Fusarium* species on annual medic pastures and reduced feed intake by sheep in Western Australia. In *Poisonous Plants: Global*

Research and Solutions, (eds KE Panter, TL Wierenga and JA Pfister). CABI Publishing, Wallingford, pp. 313-317.

Barbetti, M.J., Fang, C.S. 1991. Relationship between Phoma black stem severity and seed yield and coumestrol content in three *Medicago polymorpha* var. *brevispina* cultivars. *Australian Journal of Agricultural Research* 42, 409-415.

Barbetti, M.J., Gillespie, D.J. and Collins, W.J. 1991. Effect of *Kabatiella caulivora* isolates and host growth stage upon symptom expression and resistance in *Trifolium subterraneum*. *Australian Journal of Experimental Agriculture* 31, 63-69.

Barbetti, M. J. and Jones, R.A.C. 1999. Fungal and viral diseases limiting productivity of annual pasture legumes in a Mediterranean-type environment. In *Proceedings of Fourth International Herbage Seed Conference – Herbage Seed as a Key Factor for Improving production and Environmental Quality*. University of Perugia: Italy. pp. 69-73.

Barbetti, M.J., Jones, R.A.C. and Riley, I.T. 1996. Problems and progress in assessing direct and indirect yield losses caused by pathogens in pasture species. Pages 63-91 *In*: Pasture and Forage Crop Pathology. Eds Chakraborty, S., Leath, K.T.L., Skipp, R.A., Pederson, G.A., Bray, R.A., Latch, G.C.M. and Nutter, F.W. ASA, CSSA, and SSSA, Madison, WI.

Barbetti, M.J., Jones, R.A.C. and Stanton, J. 1989. Diseases and their control in lucerne. Western Australian Department of Agriculture, Farmnote No. 79/89.

Barbetti, M.J. and Khan, T.N. 1987. Cross-pathogenicity of *Phoma medicaginis* isolates from the genera *Medicago*, *Pisum* and *Trifolium*. *Phytophylactica* 19, 517-519.

Barbetti, M.J. and MacNish, G.C. 1978. Root rot of subterranean clover in irrigation areas of south-west, Western Australia. *Australian Journal of Experimental Agriculture and Animal Husbandry* 18, 426- 433.

Barbetti, M.J. and MacNish, G.C. 1983. Root rots of subterranean clover. *Journal of Agriculture, Western Australia*. 1, 9-10.

Barbetti, M.J. and MacNish, G.C. 1984. Effects of cultivation and cultural practices on subterranean clover root rot. *Australian Journal of Experimental Agriculture and Animal Husbandry* 24, 550-554.

Barbetti, M.J. and P.G.H. Nichols. 1991a. Herbage and seed yield losses in six varieties of subterranean clover from rust (*Uromyces trifolii-repentis*). *Australian Journal of Experimental Agriculture* 31,225-227.

Barbetti, M.J. and Nichols, P.G.H. 1991b. Effect of *Phoma medicaginis* and *Leptosphaerulina trifolii* on herbage and seed yield and coumestrol content of annual *Medicago* species. *Phytophylactica* 23, 223-227.

Barbetti, M.J. and Nichols, P.G.H. 1991c. Susceptibility of subterranean clover varieties to rust under controlled environment and field conditions. *Australian Journal of Experimental Agriculture* 31, 77-80.

Barbetti, M.J. and Nichols, P.G.H. 1991d. Susceptibility of subterranean clover varieties to powdery mildew under controlled environment and field conditions. *Australian Journal of Experimental Agriculture* 31, 81-84.

Barbetti, M.J. and Nichols, P.G.H. 1994. Field resistance in *Trifolium subterraneum* to *Uromyces trifolii- repentis* and *Cercospora zebrina*. *Australian Journal of Experimental Agriculture* 34, 1131-1136.

Barbetti, M.J. and Nichols, P.G.H. 1995. Resistance and associated yield losses from rust (*Uromyces trifolii-repentis)* in mid-season cultivars of *Trifolium subterraneum. Australian Journal of Experimental Agriculture* 35, 73-77.

Barbetti, M.J. and Nichols, P.G.H. 2005a. Field performance of subterranean clover germplasm in relation to severity of Cercospora disease. *Australasian Plant Pathology* 34, 197-201.

Barbetti, M.J. and Nichols, P.G.H. 2005b. New sources of resistance in *Trifolium subterraneum* to rust (*Uromyces trifolii-repentis*). *Australian Journal of Experimental Agriculture* 45, 1163-1166.

Barbetti, M.J. and Nicholas, D.A. 1997. Effect of Phoma black stem and pepper spot diseases on yield, regeneration, sward composition and phyto-oestrogen levels in grazed annual medic pastures. Proceedings of the Australasian Plant Pathology Society Biennial Conference, Perth, 1997, p.65.

Barbetti, M.J., Riley, I.T., You, M.P., Li, H. and Sivasithamparam, K. 2006. The association of necrotrophic fungal pathogens and plant parasitic nematodes with the loss of productivity of annual medic-based pastures in Australia and options for their management. *Australasian Plant Pathology* 35, 691-706.

Barbetti, M.J., Si, P. and Nichols, P.G.H. 2005. Genetic basis for and inheritance of resistance to Race 1 and Race 2 of *Kabatiella caulivora* in *Trifolium subterraneum* ssp. *subterraneum* and ssp. *yanninicum*. *Euphytica* 144, 237-246.

Barbetti, M.J., Sivasithamparam, K. 1986. Fungal foliar diseases of subterranean clover. *Review of Plant Pathology* 65, 513-521.

Barbetti, M.J., Sivasithamparam, K. and Wong, D.H. 1986a. Root rot of subterranean clover. *Review ofPlant Pathology* 65, 287-295.

Barbetti, M.J. and Sivasithamparam, K. 1987. Effects of soil pasteurization on root rot, seedling survival and plant dry weight of subterranean clover inoculated with six fungal root pathogens. *Australian Journal of Agricultural Research* 38, 317-327.

Barbetti, M.J., Sivasithamparam, K. and Wong, D.H. 1987a. Fungicidal drenches for control of root rot in subterranean clover. *Plant and Soil* 101, 151-157.

Barbetti, M.J., Sivasithamparam, K. and Wong, D.H. 1987b. Fungicidal seed treatments for control of root rot in subterranean clover. *Phytophylactica* 19, 57-60.

Barbetti, M.J., Sivasithamparam, K., Riley, I.T. and You, M.P. (2005). Role and impact of diseases caused by soil-borne plant pathogens in reducing productivity in southern Australian pasture systems. Meat and Livestock Australia, 80pp.

Barbetti, M.J., Wong, D.H., Sivasithamparam, K. and D'Antuono, M.F. 1986b. Response of subterranean clover cultivars to root rot fungi. *Annals of Applied Biology* 109, 259-267.

Barbetti, M.J., You, M.P., Li, H., Ma, X. and Sivasithamparam, K. 2007. Management of root diseases of annual pasture legumes in Mediterranean ecosystems – a case study of subterranean clover root diseases in the south-west of Western Australia. *Phytopathologia Mediterranea* 46, 239-258.

Barnett, O.W., Diachun, S. 1986. Virus diseases of clovers:aetiology and crop losses. In: Viruses infecting forage legumes,Vol III, eds Edwardson,J.R., Christae, R.G. pp. 625-675. University of Florida: Gainsville.

Barnett, O.W., Gibson, P.B. 1975. Identification and prevalence of white cover viruses and the resistance of Trifolium species to these viruses. *Crop Science* 15, 32-37.

Bayliss, K., Kuo, J., Sivasithamparam, K., Barbetti, M.J. and Lagudah, E.S. 2001a. Infection of subterranean clover (*Trifolium subterraneum*) by *Kabatiella caulivora*. *Journal of Phytopathology* 149, 699-705.

Bayliss, K., Kuo, J., Sivasithamparam, K., Barbetti, M.J. and Lagudah, E.S. 2001b. Variability of phenylalanine ammonia-lyase and peroxidase activities in leaves of subterranean clover is determined by their susceptibility to *Kabatiella caulivora*. *Phytopathologia Mediterranea* 40, 217-223.

Bayliss, K., Kuo, J., Sivasithamparam, K., Barbetti, M.J. and Lagudah, E.S. 2002. Differences in symptom development in subterranean clover infected with *Kabatiella caulivora* Race1 and Race 2 are related to host resistance. *Australian Journal of Agricultural Research* 53, 305-310.

Bailiss KW, Ollennu LAA (1986) Effect of alfalfa mosaic virus isolates on forage yield of lucerne(*Medicago sativa*) in Britain. *Plant Pathology* **35**, 162-168.

Bayliss, K., Spindler, L., Lagudah, E.S., Sivasithamparam, K. and Barbetti, M.J. 2003. Variability within *Kabatiella caulivora* Race 1 and Race 2 revealed by cultural and molecular analysis. *Australian Journal of Agricultural Research* 54, 77-84.

Beale, P.E. 1972. Clover wilt threat to pastures. *Journal of Agriculture, South Australia* 75: 69-71.

Beale, P.E. 1976. *Kabatiella* research - a progress report. *Journal of Agiculture, South Australia* 79, 49- 52.

Beale, P.E. 1980. Genetic variation in resistance to *Kabatiella caulivora* in *Trifolium subterraneum* subspecies *yanninicum*. Ph.D. Thesis, University of Western Australia.

Beale, P.E. and Goodchild, N.A. 1980. Genotype x environment interactions for resistance to *Kabatiella caulivora* in *Trifolium subterraneum* subspecies *yanninicum*. *Australian Journal of Agricultural Research* 31, 1111-1117.

Beale, P.E. and Thurling, N. 1979. Genotypic variation in resistance to *Kabatiella caulivora* in *Trifolium subterraneum* subspecies *yanninicum. Australian Journal of Agricultural Research* 30, 651-658.

Beale, P.E. and Thurling, N. 1980a. Reaction of *Trifolium subterraneum* genotypes to different isolates of *Kabatiella caulivora. Australian Journal of Agricultural Research* 31, 89-94.

Beale, P.E., and Thurling, N. 1980b. Genetic control of resistance to *Kabatiella caulivora* in *Trifolium subterraneum* subspecies *yanninicum*. I. Diallel analysis of variation in disease development parameters. *Australian Journal of Agricultural Research* 31, 927-933.

Beale, P.E. and Thurling, N. 1980*c*. Genetic control of resistance to *Kabatiella caulivora* in *Trifolium subterraneum* subspecies *yanninicum*. II. Variation in segregating populations. *Australian Journal of Agricultural Research* 31, 935-942.

Behncken, G.M. 1966. Lucerne mosaic virus in Queensland. *Queensland Journal of Argricultural and Animal Science* 23, 91-92.

Behncken, G.M., Gowanlock, D.H. 1976. Association of a bacterium-like organism with rugose leaf curl disease of clovers. *Australian Journal of Biological Science*. 29, 137-46.

Belotti, W. 1998. Medic Decline Syndrome - Problem Definition. GRDC Final report June 1998. Blackstock, J.M. 1978. Lucerne transient streak and lucerne latent, two new viruses of lucerne. Australian Journal of Agricultural Science 29, 291-304.

Bokor, A. 1972. Scorch disease of sub-clover. *Dairy Notes* 9, 3-5.

Bokor, A. 1982. Cercospora leaf spot of subterranean clover. Department of Agriculture, Western Australia, Farmnote No. 57/82.

Bokor, A. 1983. *Cercospora zebrina* found on *Trifolium subterraneum* L. in Western Australia. *Australasian Plant Pathology* 12, 24.

Bokor, A. and Chatel, D.L. 1973. Learning to live with clover scorch. *Journal of Agriculture, Western Australia* 14, 179-81.

Bokor, A., Chatel, D.L. and Nicholas, D.A. 1978a. Causes and symptoms of clover scorch. Dept. epartment of Agriculture Western Australia. Farmnote 68/78.

Bokor, A., Chatel, D.L. and D.A. Nicholas. 1978b. Progress in clover scorch research. *Journal of Agriculture Western Australia* 19, 54-58.

Bokor, A., Chatel, D.L. and Nicholas, D.A. 1978b. Progress in clover scorch research. *Journal of Agriculture, Western Australia* 19, 54-58.

Bolland, M., Cox, W. and Codling, B. 2001. Soil and tissue tests to predict pasture yield responses to applications of potassium fertilizer in high rainfall areas of South Western Australia. University of Western Australia.

Boswell, K.F., Gibbs, A.J. 1983. viruses of Legumes, 1983. Descriptions and Keys from VIDE. Canberra : Australian National University, Research School of Biological Sciences. I39 pp.

Bowyer, J.W., Atherton, J.G., Teakle, D.S. and Ahern, G.A. 1969. Mycoplasma-like bodies in plants affected by legume little leaf, tomato big bud and lucerne witches' broom diseases. *Australian Journal of Biological Science* 22, 271-4.

Braverman, S.W. 1986. Disease resistance in cool season forage range and turf grasses. II. *TheBotanical Review* 52, 2-112.

Bretag, T.W. 1985. Fungi associated with root rots of annual *Medicago* spp. in Australia. *Transactions of the British Mycological Sociey* 84, 329-334.

Bretag, T.W. and Kollmorgen, J.F. 1986. Effects of trifluralin, benomyl and metalaxyl on the incidence and severity of root disease in annual *Medicago* spp., and evaluation of cultivars for resistance to root rot. *Australian Journal of Experimental Agriculture* 26, 65-70.

Brink, G.E., McLaughlin, M.R. 1990. Influence of seeding rate and interplanting with tall fescue on virus infection of white clover. *Plant Disease* 74, 51-53.

Brink, G.E., McLaughlin, M.R., Pratt, R.G., Windham, G.L., Pederson, G.A. 1996. Disease interactions in legume-grass swards. In Identifying Germplasm for Successful Forage Legume-Grass Interactions, pp. 50-57. Eds T L Springer and R N Pittman. Seattle, Washington State: USDA.

Buchen-Osmond C., Crabtree K., Gibbs A., McLean G. 1988. Viruses of plants in Australia. (Australian National University Printing Service: Canberra)

Burgess, L.W., Ogle, H.J., Edgerton, J.P., Stubbs, L.L. and Nelson, P.E. 1973. The biology of fungi associated with root rot of subterranean clover in Victoria. *Proceedings of the Royal Society of Victoria* 86, 19-29.

Burnett, V.F., Coventry, D.R., Hirth, J.R., Greenhalgh, F.C. 1994. Subterranean clover decline in permanent pastures in north-eastern Victoria. *Plant and Soil* 164, 231-241.

Butler, F.C. 1953. Anthracnose and sooty blotch of red clover. *Agricultural Gazette, New South Wales* 64: 368-70, 386.

Campbell, C.L. 1984. Yield responses of six white clover clones to virus infection under field conditions. Plant Disease 68, 1033-1035.

Carver, M. 1984. *Metopolophium dirhodum* (Walker) (Homoptera: Aphididae). Newly recorded from Australia. *Journal of the Australian Entomology Society* 23, 192.

Catherall, P.L. 1978. Virus diseases of cereals and grasses and their control through plant breeding. *Report of the Welsh Plant Breeding Station 1978,* 205-226.

Catherall, P.L. 1984. Grass and cereal viruses. Welsh Plant Breed. Stn (Aberystwyth) Annual Report p.64-5.

Catherall, P.L., Parry, A.C. 1987. Effects of BYDV on some varieties of Italian, hybrid and perennial ryegrasses and their implications for grass breeders. *Plant Pathology* 36, 148-153.

Chambers, S.C. 1959. Diseases recorded on cereals, grasses and pasture legumes in Western Australia. *Journal of Agriculture, Western Australia* 7, 927-34.

Chandrashekar, M. and Halloran, G.M. 1990. Variation in, and inheritance of, seedling and adult plant resistance in subterranean clover (*Trifolium subterraneum* L.) to leaf scorch (*Kabatiella caulivora* (Kirch.) Karak*.). Australian Journal of Agricultural Research* 41, 115-120.

Chandrashekar, M. and Halloran, G.M. 1992. Leaf scorch (*Kabatiella caulivora* (Kirch.) Karak.) of subterranean clover (*Trifolium subterraneum* L.): Influence of host cultivar, growth stage and pathogen isolates on the disease severity. *Euphytica* 61, 181-186.

Chatel, D.L. and Francis, C.M. 1974*a*. Susceptibility of subterranean clover to clover scorch (*Kabatiella caulivora*). *Journal of the Australian Institute of Agricultural Science* 40, 80-82.

Chatel, D.L. and Francis, C.M. 1974*b*. The reaction of a number of varieties of subterranean clover to the clover scorch disease [*Kabatiella caulivora* (Kirchn.) Karak] at three sites in Western Australia. Department of Agriculture Western Australia,Tech. Bulletin, No. 25, 16pp.

Chatel, D.L. and Francis, C.M. 1975. Sources of variation in the resistance of subterranean clover to clover scorch (*Kabatiella caulivora*) in the field. *Australian Journal of Experimental Agriculture and Animal Husbandry* 15, 541-544.

Chatel, D.L. and Francis, C.M. 1977. The reaction of varieties of subterranean clover to clover scorch disease [*Kabatiella caulivora* (Kirchn.) Karak] at Denmark, Western Australia in 1974 and 1975. Research Report unnumbered. Department of Agricultre Western Australia, Plant Research Division, 24pp.

Chatel, D.L. and Francis, C.M. 1978. The reaction of varieties of subterranean clover to clover scorch disease [*Kabatiella caulivora* (Kirchn.) Karak] at Denmark, Western Australia in 1976 and 1977. Research Report unnumbered. Department of Agricultre Western Australia, Plant Research Division, 42pp.

Chatel, D.L., Francis, C.M. and Devitt, A.C. 1973. Varietal variation in resistance to clover scorch [*Kabatiella caulivora* (Kirch.) Karak] in *Trifolium subterraneum L.* Department of Agricultre Western Australia, Technical Bulletin 17, 1-11.

Chatel, D.L., Bokor, A. and Devitt, A.C. 1972. Some agronomic aspects of clover scorch disease in Western Australia. Department of Agriculture, Western Australia, Technical Notes 72, 1-8.

Chu, P.W.G., Anderson B.J., Khan. M.R.I. et al. 1999. Production of bean yellow mosaic virus resistant subterranean clover (*Trifolium subterraneum*) plants by transformation with the virus coat protein gene. *Annals of Applied Biology* 135, 469-480.

Chu, P.W.G., Vetten, H.J. 3003. Subterrnean clover stunt virus. Descriptions of plant viruses No. 396. AAB: Wellesboune UK.

Clarke, R. 1980. Clover scorch disease of subterranean clover. Department of Agriculture, Victoria.Agnote 1211/80.

Clarke, R. 1981a. Diseases of lucerne 2: fungus diseases of leaves and stems.Department ofAgriculture, Victoria, Agnote 1532/81.

Clarke, R. 1981b. Diseases of lucerne 3: fungus diseases of roots and crowns.Department of Agriculture, Victoria, Agnote 1533/81.

Clarke, R. 1983a. Diseases of white clover - 1. Department of Agriculture, Victoria, Agnote 2045/83.

Clarke, R. 1983b. Root rot of subterranean clover. Department of Agriculture, Victoria, Agnote 137/633.

Clarke, R. 1999a. Diseases of lucerne. Vol 3: Fungal root and crown diseases. Agriculture Victoria Notes Series No. AG0728.

Clarke, R. 1999b. Diseases of Lucerne - 2: Fungal Leaf Diseases. Note Number: AG0727. Department of Primary Industries, Victoria, Australia

Clarke, R. 1999c. Diseases of white clover - 1: virus diseases.

Clarke, R. 1999d. Diseases of white clover - 2: Fungal diseases.

Clarke, R. 1999e. Diseases of white clover - 3: Leaf spot diseases.

Clarke, R.G. and Eagling, D.R. 1994. Effects of pathogens on perennial pasture grasses. *New Zealand Journal of Agriculural Research* 37, 319-327.

Clarke R.G. and Greenhalgh, F.C. 1986. Screening for alternative hosts of *Phytophthora clandestina*. *Australasian Plant Pathology* 15, 61-63.

Colman, R.L. 1964. Root knot nematodes as a cause of poor growth of clover on the north coast of N.S.W. *The Agricultural Gazette, New South Wales* 75, 136-138.

Cook, F.G. 1975. Production loss estimation in *Drechslera* infection of ryegrass. *Annals of Applied Biology* 81, 251-256.

Cotterill, P.J. and Sivasithamparam, K. 1988. Importance of the proportion of grassy weeds within legume crops in the perpetuation of *Gaeumannomyces graminis* var. *tritici*. *Plant Pathology* 37, 337-343.

Cocks, P.S., Mathison, M.J.. Crawford, EJ. 1978. From wild plants to pasture cultivars: annual medics and subterranean clover in southern Australia. In 'Advances in Legume Science'. Vol. I, p. 569-96. (Proceedings of the International Legume Conference, Kew 1978. (Eds Summerfield, R.J. and Bunting, A.H.). Royal Botanic Gardens, Kew, England.)

Coutts, B. A. ,Jones, R. A. C. 1999. Incidence of four viruses in white clover-based pastures over an extended period. In Proceedings of 15th Meeting of the International Working Group on Legume Viruses, Perth Western Australia, August 15-17, 1999.

Coutts, B. A., Jones, R. A. C. 1999. Incidence of four viruses in white clover-based pastures over an extended period. In Proceedings of 7th Australasian Conference on Grassland Invertebrate Ecology. Ed. J N Matthiessen. CSIRO: Perth, Western Australia, p. 297.

Coutts, B.A., Jones, R.A.C. 2000. Viruses infecting canola (*Brassica napus*) in south-west Australia: incidence, distribution, spread and infection reservoir in wild radish (*Raphanus raphanistrum*). *Australian Journal of Agricultural Research* 51: 925-936.

Coutts B.A., Jones R.A.C. 2002. Temporal dynamics of spread of four viruses within mixed species perennial pastures. *Annals of Applied Biology* 140, 37-52.

Coutts, B.A., Banovic, M, Kehoe, M.A., Jones, R.A.C 2010. Wheat streak mosaic virus: alternative hosts, infection of wheat, oat and barley cultivars, seed transmission studies and spatial and temporal spread patterns. In: 9th Australasian Plant Virology Workshop, Melbourne, 16-19 November 2010. p. 69.

Coutts, B.A., Hawkes, J. R., Jones, R.A.C. 2006. Occurrence of Beet western yellows virus and its aphid vectors in over-summering broad-leafed weeds and volunteer crop plants in the grainbelt region of south-western Australia. *Australian Journal of Agricultural Research* 57, 975-982.

Coutts, B.A., Hammond, N.E.B., Kehoe, M.A., Jones, R.A.C. 2008. Finding Wheat streak mosaic virus in south-west Australia. *Australian Journal of Agricultural Research* 59, 836-843.

Coutts, B., Strickland, G, Kehoe, M. Severtson, D. (2008). Epidemiology studies on Wheat streak mosaic virus in 2007. Cereal Updates 2008 (ed. S. Penny), Agribusiness Crop Updates, Burswood Entertainment Complex, Perth, WA. 13-14, February, pp. 83-85.

Coutts, B.A., Strickland, G.R., Kehoe, M.A., Severtson, D.L., Jones, R.A.C. 2008. The epidemiology of Wheat streak mosaic virus in Australia: case histories, gradients, mite vectors, and alternative hosts. *Australian Journal of Agricultural Research* 59, 844-853.

Crill P, Hagedorn DJ, Hanson EW (1970) Incidence and effect of alfalfa mosaic virus on alfalfa. Phytopathology 60, 1432-1435.

Croker, K., Barbetti, M.J. and Nichols, P.G.H. 1994a. Incidence of coumestrol in medic pastures in Western Australia. *Proceedings of the Australian Society of Animal Production* 20, 416.

Croker, K., Nichols, P.G.H., Barbetti, M.J. and Adams N. 1994b. Sheep infertility from pasture legumes. Department of Agriculture, Western Australia, Farmnote No. 6/94.

Croker, K., Nichols, P.G.H., Barbetti, M.J. and Adams N. 1999. Sheep infertility from pasture legumes. Department of Agriculture, Western Australia, Farmnote No. 79/99.

Croker, K., Nichols, P.G.H., Barbetti, M.J. and Adams N. 2005. Sheep infertility from pasture legumes. Department of Agriculture, Western Australia, Farmnote 41/2005.

Dall. D.J., Randles, J.W., Francki, R./I.B. 1989, The effect of alfalfa mosaic virus on productivity of annual barrel medic, *Medicago truncatula*. *Australian Journal of Agricultural Research* 40, 807-815.

Dear, B.S., Moore, G.A., Hughes, S.J. 2003. Adaptation and potential contribution of temperate perennial legumes to the southern Australian wheatbelt: a review. *Australian Journal of Experimental Agriculture* 43, 1-18.

Dear, B.S., Murray, G.M., Cregan, P.D. and Taylor, P.A. 1993. Comparison of the performance of subterranean clover cultivars in southern New South Wales. 2. Effects of *Phytophthora clandestina* and bromoxynil on seedling survival, growth, and seed set. *Australian Journal of Experimental Agriculture* 33, 591-596.

Dewan, M.M. and Sivasithamparam, K. 1988. A plant growth promoting sterile fungus from wheat and ryegrass roots with potential for suppressing take-all. *Transactions of the British Mycological Society* 91, 687-717.

Dewan, M.M. and Sivasithamparam, K. 1990a. Effect of colonization by a sterile red fungus on the viability of seed and growth and anatomy of wheat roots. *Mycological Research* 94, 553-557.

Dewan M.M. and Sivastihamparam, K. 1990b. Differences in the pathogenicity of isolates of the take-all fungus from roots of wheat and ryegrass. *Soil Biology and Biochemistry* 22, 119-122.

Dewan M.M. and Sivastihamparam, K. 1998a. *Pythium* spp. in roots of wheat and ryegrass in Western Australia and their effect on root rot caused by *Gaeumannomyces graminis* var. *tritici*. *Soil Biology and Bioichemistry* 20, 801-808.

Dewan M.M. and Sivastihamparam, K. 1998b. Identity and frequency of occurrence of *Trichoderma* spp. in roots and wheat of ryegrass in Western Australia and their effect on root rot caused by *Gaeumannomyces graminis* var. *tritici*. *Plant and Soil* 109, 93-101.

Dewan M.M. and Sivastihamparam, K. 1998c. Occurrence of species of *Aspergillus* and *Penicillium* in roots of wheat and ryegrass and their effect on root rot caused by *Gaeumannomyces graminis* var. *tritici*. *Australian Journal of Botany* 36, 701-710.

Dewan M.M. and Sivastihamparam, K. 1998d. A plant growth promoting sterile fungus from wheat and ryegrass roots with potential for suppressing take-all. *Transactions of the British Mycological Society* 91, 687-692.

Dwyer, G.I., Gibbs, M.J., Gibbs, A.J., Jones, R.A.C. 2007. *Wheat streak mosaic virus* in Australia: relationship to isolates from the Pacific Northwest of the USA and its dispersion via seed transmission. *Plant Disease* 91, 164-170.

Dwyer, G.I., Njeru, R., Williamson, S., Fosu-Nyarko, J., Hopkins, R., Jones, R.A.C., Waterhouse, P.M., Jones, M.G.K. 2003. The complete nucleotide sequence of *Subterranean clover mottle virus*. *Archives of Virology* 148, 2237-2247.

Eagling, D.R., Cunningham, P.J., Sward, R.J., Hallorgan, G.M. 1989a. The incidence of barley yellow dwarf virus isolates in perennial ryegrass (*Lolium perenne*) in south-western Victoria. *Plant Pathology* 38, 408-413.

Eagling, D.R., Cunningham, P.J., Sward, R.J., Halloran, G.M. 1989a. The incidence of barley yellow dwarf virus isolates in perennial ryegrass (*Lolium perenne)* in south-western Victoria. *Plant pathology* 38, 408-413.

Eagling, D.R., Hallorgan, G.M., Sward, R.J., 1989b.The effect of barley yellow dwarf virus on early plant growth in ryegrass (*Lolium* spp.). *Australian Journal of Agricultural Research* 40, 293-29.

Eagling, D.R., Sward, R.J. 1989. The effect of viruses on the quality of pastures. *In:* The feed quality of pastures. *Department of Agricultural and Rural Affairs Pasture Specialists Conference, Wodonga* 1989*,* 95-96.

Eagling, D.R., Villatta, O., Sward, R.J. 1992. Host range, symptoms and effects on pasture production of a Victorian isolate of ryegrass mosaic potyvirus. *Australian Journal of Agricultural Research* 43, 1243-1251.

Eagling, D.R., Villatta, O., Hallorgan, G.M. 1993. Sources of resistance to barley yellow dwarf luteovirus in perennial ryegrass (*Lolium perenne*). *Australian Journal of Agricultural Research* 44, 33-40.

Edwards, E.T. 1932. Stem nematode disease of lucerne, with review of literature concerning the causal organism *Tylenchus dipsaci* (Kuhn) Bast. *Agricultural Gazette of New South Wales* 43, 305-314 and 345-356.

Edwardson JR, Christie RG (Eds) (1986) 'Viruses infecting forage legumes.', Vols 1-3, 742p. (University of Florida Agricultural Experimental Station: Gainesville, FL).

Ellis, M.H., Rebetzke, G.J., Mago, R., Chu, P.R. 2003. First report of Wheat streak mosaic virus in Australia. *Australasian Plant Pathology* 32, 551-553.

Ellis, M.H., Rebetzke, G.J., Kelman, W.M., Moore, C.S., Hyles, J.E. 2004. Detection of Wheat streak mosaic virus in four pasture grass species in Australia. *Plant Pathology* 53, 239.

Ferris, D.G., Jones R.A.C. 1994. Virus diseases of subterranean clover pastures and their management. *Journal of Agriculture - Western* Australia, Fourth Series 35, 38-44.

Ferris, D.G., Jones, R.A.C. 1995. Losses in herbage and seed yields caused by subterranean clover mottle sobemovirus in grazed subterranean clover swards. *Australian Journal of Agricultural Research* 46, 775-791.

Ferris, D.G., Jones R.A.C. 1996. Natural resistance to bean yellow mosaic potyvirus in subterranean clover. *Australian Journal of Agricultural Research* 47, 605-624.

Ferris, D.G., Jones, R.A.C., Wroth, J.M. 1996. Determining the effectiveness of resistance to subterranean clover mottle sobemovirus in different genotypes of subterranean clover in the field using the gazing animal as virus vector. *Annals of Applied Biology* 128, 303-315.

Fletcher, M. 1980. Yellows. Research Report 79. NSW, Australia: New South Wales Department of Agriculture: Biological and Chemical Research Institute.

Flett, S.P. 1994. Studies on *Phytophthora clandestina*, the cause of taproot rot in subterranean clover 1.Evidence for physiological specialisation in *Phytophthora clandestina*. *Australian Journal of Experimental Agriculture* 34, 1125–1129

Flett, S.P., Clarke, R.G. Disease complexes in Australian pastures. Pages 403-427 *In*: Pasture and Forage Crop Pathology. Eds S. Chakraborty, K.T.L. Leath, R.A. Skipp, G.A. Pederson, R.A. Bray, G.C.M. Latch, G.C.M. and F.W. Nutter. ASA, CSSA, and SSSA, Madison, WI.

Forster, R.L.S., Jones, A.T. 1980. Lucerne transient strak virus. CMI/AAB Descriptions of Plant Viruses, No. 224.

Forster, R.L.S., Jones, A.T. 1979. Properties of lucerne transient streak virus and evidence of its affinity to southern bean mosaic virus. *Annals of Applied Biology* 93 181-189.

Fosu-Nyarko, J., Jones, R.A.C., Dwyer, G.I., Jones, M.G.K. 2005. Subterranean clover mottle virus, a pathogen of pasture legumes. In Proceedings of 2nd Model Legume Workshop – Application to Crop and pasture improvement, Rottnest Island, WA, 5-8 April 2005. p.58.

Fosu-Nyarko, J., Jones R.A.C., Smith L.J., Jones, M.G.K., Dwyer, G.I. 2002. Host range and symptomatology of *Subterranean clover mottle virus* in alternative pasture, forage and crop legumes. *Australasian Plant Pathology* 31, 345-350.

Fraser, J., McCartney, D., Najda, H. and Mir, Z. 2004. Yield potential and forage quality of annual forage legumes in southern Alberta and northeast Saskatchewan. *Canadian Journal of Plant Sciences*84, 143-155.

Francki, R.I.B, Hatta, T. 1980. Chloris striate mosaic virus. CMI/AAB Descriptions of Plant Viruses, No.221.

Francki, R.I.B., Mossop, D.W., Hatta, T. 1979. Cucumber mosaic virus. CMI/AAB Descriptions of Plant Viruses No. 213.

Francki, R.I.B., Randles, J.W., Hatta, T., Davies, C., Chu, P.W.G., McLean, G.D. 1983. Subterranean clover mottle viru : another virus from Australia with encapsidated viroid-like RNA. *Plant Pathology* 32, 47 - 59.

Freeman, A.J., Aftab, M. 2011. Effective management of viruses in pulse crops in south eastern Australia should include management of weeds. *Australasian Plant Pathology* 40, 430-441.

Frosheiser FI (1969) Variable influence of alfalfa mosaic virus strains on growth and survival of alfalfa and on mechanical and aphid transmission. *Phytopathology* 59, 857-862.

Frost, W.E. 1993. Aspects of the population ecology of *Abacarus hystrix* Nalepa in South Australian dairy pastures. pp. 388-390 *in:* Pest control and sustainable agriculture, Corey, S., Dall, D., Milne.W. *ed.* CSIRO, Canberra.

Frost, W.E., Eagling, D.R., Mason, D.C.M. 1990. *Abacarus hystrix* (Nalepa) (Acarina: Eriophyidae): newly recorded in Australia. *Journal of the Australian Entomological Society* 29, 182

Garran, J., Gibbs, A.J. 1982. Studies of alfalfa mosaic virus and alfalfa aphids. *Australian Journal of Agricultural Research* 33, 657 - 664.

Garrett, R.G. 1983. Confirmation of the presence of clover yellow vein virus in Victoria. Proceedingsof the 4th International Congress of Plant Pathology, p. 115.

Garrett, R.G. 1985. Cucumber mosaic virus in pasture legumes. Victorian Department of AgricultureCrop Information Service Field Crop and Pasture Protection Bulletin No. 58, p. 13.

Garrett, R.G. 1991. Impact of viruses on pasture legume productivity. In "Proceedings of Department of Agriculture Victoria White Clover Conference", pp. 50-56.

Geard I.D. 1960. Red leaf of oats. A virus disease of cereals in Tasmania. *Tasmanian Journal of Agriculture* 31-320-324.

Gibson, P.B., Barnett, O.W. 1977. Identifying virus resistance in white clover by applying strong selection pressure. II. Screening program. Proc. 34th Southern USA Pasture and Forage Crop Conference, pp. 74-79.

Gibson, R.W., Heard, A.J. 1979. The resistance of two clones of perennial ryegrass and their progeny to ryegrass mosaic virus from different parts of Britain. *Annals of Applied Biology* 91, 101-106.

Gibson, P.B., Barnett, O.W., Burrows, P.M., King, F.D. 1982. Filtered-air enclosures exclude vectors and enable measurement of effects of viruses on white clover in the field. *Plant Disease* 66, 142-144.

Gillespie, D.J. 1979. Western Australian Department of Agriculture, Annual Report 1978/79, pp. 29-30. Gillespie, D.J. 1980. Western Australian Department of Agriculture, Annual Report 1979/80. pp. 40-44. Gillespie, D.J. 1981. Meteora - a new clover for long growing season environments. Department ofAgricultre Western Australia, Farmnote 67/81.

Gillespie, D.J. 1983a. Western Australian Department of Agriculture, Annual Report 1982/83. pp. 78-81. Gillespie, D.J. 1983b. Developing clovers for disease and insect resistance. *Journal of Agriculture, Western Australia* 1, 14-15.

Gillespie, D.J. 1983c. Pasture deterioration - causes and cures. *Journal of Agriculture, Western Australia* 1, 3-8.

Gladstones, J.S., Collins, W.J. 1983. Subterranean clover as a naturalized plant in Australia. *Journal of the Australian Institute of Agricultural Science* 49, 191 - 202.

Godfree, R. C., Chu, P.W.G., Woods, M.J. 2004. White clover (*Trifolium repens*) and associated viruses in the subalpine region of south-eastern Australia: implications for GMO risk assessment. *Australian Journal of Botany* 52, 321 – 331.

Goodchild, D.J. 1956a. Relationships of legume viruses in Australia. I. Strains of bean yellow mosaic virus and pea mosaic virus. *Australian Journal of Biological Sciences* 9, 213 - 230.

Goodchild, D.J. 1956b. Relationships of legume viruses in Australia. 11. Serological relationships of bean yellow mosaic virus and pea mosaic virus. *Australian Journal of Biological Sciences* 9, 231 - 231.

Graddon, D.J., McLean, G.D., Randles, J.W. 1984. Geographical distribution and variation in the viroid- like RNA associated with subterranean clover mottle virus. Proceedings of the VIth International Congress of Virology, p. 384.

Gramshaw, D. 1981. Factors limiting lucerne production. *Queensland Agricultural Journal* 107, 25-8. Gramshaw, D., Langdon, P.W., Lowe, K.F., Lloyd, D.L. 1985.Performance of North American andAustralian lucernes in the Queensland sub-tropics. 1. Field reaction to important root and crown diseases. *Australian Journal of Experimental Agriculture* 25, 76-81.

Greber, R.S., 1979. Digitaria striate virus – a rhabdovirus of grasses transmitted by Sogatella kolopon(Kirk.). *Australian Journal of Agricultural Research* 30, 43-51.

Greber, R.S. 1988. Ecology of barely yellow dwarf virus in south-east Queensland. *Australasian Plant Pathology* 17, 101-104.

Greenhalgh, F.C. 1983. Growth cabinet evaluation of fungicides for control of Pythium damping-off and root rot of subterranean clover. *Fungicide and Nematicide Tests* 38, 47.

Greenhalgh, F.C. 1992. Studies on the etiology and significance of root diseases of subterranean clover and the histopathology and control of Phytophtora root rot. PhD Thesis, La Trobe University, Victoria, Australia.

Greenhalgh, F.C. and Clarke, R.G. 1985. The use of fungicides to study the significance and etiology of root rot of subterranean clover in dryland pastures of Victoria. Pages 234-236 *In*: Ecology and Management of Soil-borne Plant Pathogens. Eds. Parker, C.A., Moore, K.J., Wong, P.T.W., Rovira, A.D. and J.F. Kollmorgen. St Paul, U.S.A.; American Phytopathological Society.

Greenhalgh, F.C., de Boer, R.F., Merriman, P.R., Hepworth, G. and Keane, P.J. 1994. Control of Phytophthora root rot of irrigated subterranean clover with potassium phosphonate in Victoria, Australia. *Plant Pathology* 43, 1009-1019.

Greenhalgh, F.C. and Flett, S. 1987. Recent research on root diseases of subterranean clover in Victoria and its implications. *In*: National Subterranean Clover Improvement Program, Proceedings of the 3rd National Workshop. Department of Agriculture, New South Wales.

Greenhalgh, F.C. and Lucas, S.E. 1984. Effect of soil pasteurization on damping-off and root rot of subterranean clover caused by *Fusarium avenaceum* and *Pythium* spp. *Soil Biology and Biochemistry* 16, 87-88.

Greenhalgh, F.C., Merriman, P.R. and Keane, P.J. 1985. *Aphanomyces euteiches* a cause of root rot of subterranean clover in Victoria. *Australasian Plant Pathology*. 14, 34-7.

Greenhalgh, F.C. and Taylor, P.A. 1985. *Phytophthora clandestina*, cause of severe tap root rot of subterranean clover in Victoria, Australia. *Plant Disease* 69, 1002-1004.

Grylls, N.E. 1955. An outbreak of rugose leaf curl disease on clover in Queensland. *Journal of theAustralian. Institute of Agricultural Science* 21, 187-188.

Grylls, N.E., 1963. A striate mosaic virus disease of grasses and cereals in Australia, transmitted by the cicadellid *Nesoclutha obscura*. *Australian Journal of Agricultural Research* 14, 143-153.

Grylls, N.E. Butler, F.C 1956. An aphid transmitted virus affecting subterranean clover. *Journal of the Australian Institute of Agricultural Science* 22, 73 - 74.

Grylls, N.E. Butler, F.C. 1959. Subterranean clover stunt, a virus disease of pasture legumes. *Australian Journal of Agricultural Research* 10, 145 - 159.

Grylls, N.E. Day, M. 1966. Susceptibility, inhibition of infection in stolons, and induced resistance to rugose leaf curl virus infection in white clovers. *Australian Journal of Agricultural Research*. 17, 119-131.

Grylls, N.E. and Peak, J.W 1960. Varietal reactions and genetic resistance of subterranean clover (Trifolium subterraneum L.) to subterranean clover stunt virus infection. *Australian Journal of Agricultural Research* 11, 123 - 133.

Grylls, N.E. and Peak, J.W. 1969. A virus complex of subterranean clover. *Australian Journal of Agricultural Research* 20, 37 - 45.

Gutierrez, A.P., Morgan, D.I., Havenstein, D.E. 1971. The ecology of *Aphis craccivora* Koch and subterranean clover stunt virus. I. The phenology of aphid populations and the epidemiology of virus in pastures in south-east *Australian Journal of Applied Ecology* 8, 699 - 721.

Gutierrez, A.P., Havenstein, D.E., Nix, H.A., Moore, P.A. 1974. The ecology of *Aphis craccivora* Koch and subterranean clover stunt virus in south-east Australia. 11. A model of cowpea aphid populations in temperate pastures. *Journal of Applied Ecology* 11, 1 - 20.

Gutierrez, A.P., Nix, H.A., Havenstein, D.E., Moore, P.A. 1974. The ecology *of Aphis craccivora* Koch and subterranean clover stunt virus in south-east Australia. 111. A regional perspective of the phenology and migration of the cowpea aphid. Journal of Applied Ecology 11, 21 - 35.

Guy, P.L 1983. White clover mosaic virus. In Viruses in Legumes, 1983. Descriptions and Keys from VIDE, pp. 86 - 87, 114 - 115. Eds K. F. Boswell and A. J. Gibbs. Canberra: Australian National University, Research School of Biological Sciences.

Guy, P.L., Johnstone, G.R. 1985. The ecology and control of barley yellow dwarf viruses in pasture grasses. Proceedings of the 15th International Grasslands Congress, Kyoto. Japan, 780-1.

Guy, P.L 1986. Pasture ecology of barley yellow dwarf viruses at Sandford, Tasmania. *Plant Pathology* 37, 546-550.

Guy, P.L., Gibbs, A.J., Harrower, K. 1980. The effect of white clover mosaic virus on nodulation of white clover (*Trifolium repens*). Australian *Journal of Agricultural Research* 31, 307-311.

Guy, P.L., Johnstone, G.R., Duffus, J.E. 1986. The occurrence and identity of barley yellow dwarf viruses in Tasmanian pasture grasses. Australian *Journal of Agricultural Research* 37, 43-53.

Guy, P.L., Johnstone, G.R., Norris D,I. 1987. barley yellow dwarf viruses in, and aphids on, grasses(including cereals) in tasmania. *Australian Journal of Agricultural Research* 38, 139-152.

Guy, P.L., Sward, R.J. 1991. Ryegrass mosaic and ryegrass cryptic virus in Australia. Acta Phytopathologica et Entomologica Hungaricae 26, 199-202.

Haan, R.L. de., Sheaffer, C.C., Samac, D.A., Moynihan, J.M. and Barnes, D.K. 2002. Evaluation of annual *Medicago* for Upper Midwest agroecosystems. *Journal of Agronomy and Crop Science* 188, 417-425.

Haggar, R.J., Clements, R.O., Carr, A.K.H. and Peel, S. 1984. Crop Protection Handbook - Grass and Clover Swards. (Ed Williams RD). The Lavenham Press Ltd., Lavenham, Suffolk, United Kingdom.

Hajimorad MR, Francki RIB, 1988. Alfalfa mosaic virus isolates from lucerne in South Australia: biological variability and antigenic similarity. *Annals of Applied Biology* 113, 45–54.

Hannam, R.J. and Reuter, D.J. 1987. Trace element nutrition in pastures. Pages 175-190 *In*: Temperate Pastures, their Production, Use and Management. Eds J.L. Wheeler, C.J. Pearson and G.E. Robards. Australian Wool Corporation Technical Publication, Commonwealth Scientific and Industrial Research Organisation, Melbourne, Australia.

Harvey, H.L. 1956. Bean, subterranean clover and lupin diseases caused by the bean yellow mosaic virus in Western Australia. *Journal of Agriculture, Western Australia*, Third Series 5, 329–336

Harvey, H.L. 1958. A note on the occurrence of stunt disease in subterranean clover. Journal of *Australian Journal of Agricultural Research* 37 (in press)

Hawkes, J.R. and Jones, R.A.C. 2005. Incidence and distribution of *Barley yellow dwarf virus* and *Cereal yellow dwarf virus* in over-summering grasses in a Mediterranean-type environment. *Australian Journal of Agricultural Research* 56, 257-270.

Hellemere JL, 1972. Lucerne yellows. BCRI (New South Wales Department of Agriculture, Biological and Chemical Research Institute) News. NSW, Australia: New South Wales Department of Agriculture: BCRI, 6–7

Helms, K. 1975a. Development of *Kabatiella caulivora* in plants of *Trifolium subterraneum* "Yarloop" of different ages. Phytopathology 65, 197-201.

Helms, K. 1975b. Humidity, free water and light in relation to development of *Kabatiella caulivora* (Kirchn). Karak in *Trifolium subterraneum* L. cv. Yarloop. *Australian Journal of Agricultural Research* 26, 511-520.

Helms, K. 1975c. Variation in susceptibility of cultivars of *Trifolium subterraneum* to *Kabatiella caulivora* and in pathogenicity of isolates of the fungus as shown in germination-inoculation tests*. Australian Journal of Agricultural Research* 26, 647-655.

Helms, K. 1977. Effect of temperature on development of *Kabatiella caulivora* in *Trifolium subterraneum*. *Phytopathology* 67, 230-233.

Helms, K., Chu, P.W.G., Martin, R.R. 1992. Subterranean clover distortion - a new virus-like disease with unusual characteristics. *Plant Disease* 76, 420-425.

Helms, K., Muller, W.J., Waterhouse, P.M. 1987. Subterranean clover red leaf virus-disease - effects of light-intensity on plant symptoms, growth, and virus content. *Phytopathology* 77, 1297-1300.

Helms, K, Muller, W, Waterhouse, P. 1993a. National survey of viruses in pastures of subterranean clover. I. Incidence of four viruses assessed by ELISA. *Australian Journal of Agricultural Research* 44, 1837-1862.

Helms, K., Waterhouse, P.M., Muller, W.J. 1993b. *Aulacorthum (Neomyzus) circumfiexum*, a vector of subterranean clover red leaf virus. *Australasian Plant Pathology* 12, 66 - 67.

Helms, K., Waterhouse, P.M., Muller, W.J. 1985. Subterranean clover red leaf virus: effects of temperature on plant symptoms, growth, and virus content. *Phytopathology* 75, 337-341.

Helms, K., Waterhouse, P.M., Muller, W.J. 1986. Survey of viruses in subterranean clover. In Production and Utilization of Pasture in Southern Australia. Eds J. L. Wheeler, C. J. Pearson and G. E. Robards. Melbourne: CSIRO Editorial and Publishing Service.

Henry, M., Francki, R.I.B., Wallwork, H. 1992. Occurrence of barley yellow dwarf virus in cereals and grasses of the low rainfall grainbelt of South Australia. *Plant Pathology* 41, 713-721.

Hill, N.L. and Korte CJ (1998) Nutrition and disease control improve Victorian Mallee pastures. Pages 20- 23 *In*: Proceedings of the 9th Australian Agronomy Conference. Wagga Wagga, Australia, July 1998.

Hiruki C, Miczynski KA (1978) Severe isolate of alfalfa mosaic virus and its impact on alfalfa cultivars grown in Alberta. *Plant Disease* 71, 1014-1018.

Hochman, Z., Osborne, G.J., Taylor, P.A. and Cullis, B. 1990. Factors contributing to reduced productivity of subterranean clover pastures on acid soils. *Australian Journal of Agricultural Research* 41, 669-82

Hollaway, G. 2002. Root lesion nematodes. Agricultural Notes AG0846, Department of Primary Industries, Victoria, 2 pp.

Holtkamp, R.H., Clift, A.D. 1993. Establishment of three species of lucerne aphids on 24 cultivars of lucerne. *Australian Journal of Agricultural Research* 44, 53–58.

Hotton, G. 1984. Enfield - a new sub.clover cultivar. Department of Agriculture, Victoria, Australia, Agnote2607/84.

Hulse, L.B. Helms, K. Waterhouse, P. M. 1983. National survey of viruses in pastures of subterranean clover. III. Equipment for large scale screening of viruses by ELISA. . *Australian Journal of Agricultural Research* 44, 1883-1993.

Hutton, R.E., Ballard, R.A., Taylor, S.P. and Dennis, J.I. 1999. Root lesion nematodes (*Pratylenchus* spp.) cause damage to the roots of annual pasture legumes. Page 154 *In*: Proceedings of the 12th Biennial Australasian Plant Pathology Conference. Canberra, Australia, 27–30 September1999.

Hutton, E.M. and Grylls, N.E. (1956). Legume "little leaf', a virus disease of subtropical pasture species.*Australian Journal of Agricultural Research* 7, 85-97.

Hutton, E.M. and J.W. Peake. 1954. Varietal reaction of *Trifolium subterraneum* to Phaseolus virus 2 Pierce. *Australian Journal of Agricultural Research* 5, 598-607.

Irwin, J.A.G. 1974. Reaction of lucerne cultivars to *Phytophthora megasperma*, the cause of a root rot inQueensland. *Australian Journal of Experimental Agriculture and Animal Husbandry* 14, 561-565.

Irwin, J.A.G. 1976. Observations on the mode of infection of lucerne roots by *Phytophthora megasperma.Australian Journal of Botany* 24, 447-451.

Irwin, J.A.G. and Jones, R.M. 1977. The role of fungi and nematodes as factors associated with death of white clover (*Trifolium repens*) stolons over summer in south-eastern Queensland. *Australian Journal of Experimental Agriculture and Animal Husbandry* 17, 789-794.

Irwin, J.A.G., Mackie, J.M., Marney, T.S., Musial, J.M. and Robert, S. 2004. Incidence of *Stagonospora meliloti* and *Acrocalymma medicaginis* in Lucerne crowns and roots in eastern Australia, their comparative aggressiveness to lucerne and inheritance of reaction to *S. meliloti* in Lucerne. *Australasian Plant Pathology* 33, 61-67.

Johnson, B. 1957. Studies on the dispersal by upper winds of Aphis craccivora Koch in New South Wales. *Proceedings of the Linnaean Society of New South Wales* 82, 191 - 198.

Johnstone, G.R. 1981. Ecology and control of non-persistent viruses in Australia. Australasian *Plant Pathology* 10, 48 - 51.

Johnstone, G.R. 1983. The reactions of subterranean clover to inoculation with two persistent aphid transmitted viruses. *Proceedings of the International Working Group on Legume Viruses*, Binnu Burra, Queensland, 11 - 12 August, pp. 7 - 8.

Johnstone, G.R. 1985. Virus diseases of *Trifolium* species in temperate Australia. *Proceedings of the 3rd Australian. Agronomy Conference*, Hobart, pp.160.

Johnstone, G. R., Ashby, J.W., Gibbs, A.J., Duffus, J.E., Thottapilly, G., Fletcher, J.D. 1984. The host ranges, classification and identification of eight persistent aphid-transmitted viruses causing diseases of legumes. *Netherlands Journal of Plant Pathology* 90, 225 - 245.

Johnstone, G.R. and M.J. Barbetti. 1987. Impact of fungal and viral diseases on pasture. Pages 235-248 *In*: Temperate Pastures, their Production, Use and Management. Eds J.L. Wheeler, C.J. Pearson and G.E. Robards. Australian Wool Corporation Technical Publication, Commonwealth Scientific and Industrial Research Organisation, Melbourne, Australia.

Johnstone, G.R., McLean, G.D., 1987. Virus diseases of subterranean clover. Ann. Appl. Biol. 110, 421- 40.

Johnstone, G.R., Duffus, J.E. 1984. Some luteovirus diseases in Tasmania caused by beet western yellows and subterranean clover red leaf viruses. *Australian Journal of Agricultural Research* 35, 821 – 830.

Johnstone, G.R., Duffus, J.E., Munro, D., Ashby, J. W. (1982). Purification of a Tasmanian isolate of subterranean clover red leaf virus and its serological interactions with a New Zealand isolate and other luteoviruses. *Australian Journal of Agricultural Research* 33, 697 - 703.

Johnstone, G.R., Chu, P.W.G. 1993. Viral diseaes of white clover. In: 'White clover: a key to increasing milk yields'. Ed. W. Mason. Pp-83-86. Dairy Research and Development Corporation.

Johnstone, G.R., Patten, D.S. 1981. Non-transmission of sub-clover red leaf and sub-clover stunt viruses by lucerne and pea aphids. *Australasian Plant Pathology* 10, 65 - 66.

Jones, A.T. 1983. Lucerne Australian latent virus. In Viruses of Legumes, 1983. Descriptions and Keys from VIDE, pp. 62 - 63, 101. Eds K.F. Boswell and A.J. Gibbs. Canberra: Australian National University, Research School of Biological Sciences

Jones, A.T., Forster, R.L.S. 1980. Lucerne Australian latent virus. CMI/AAB Descriptions of Plant Viruses, No. 225.

Jones, A.T., Forster, R.L.S., Mohamed, N.A. 1979. Purification and properties of Australian lucerne latent virus, a seed-borne virus having affinities with nepoviruses. *Annals of'Applied Biology* 92, 49-59.

Jones, D.G., Heard, A.J., Woledge, J., Leafe, E.L., Plumb, R.T. 1977b. The effect of ryegrass mosaic virus on carbon assimilation and growth of ryegrasses. *Annals of Applied Biology* 87, 393-405.

Jones, R.A.C. 1987. Seed-transmitted viruses of grain and pasture legumes in Western Australia. Abstracts of Conference on Viruses with Fungal Vectors, Association of Applied Biologists, St Andrews, Scotland. p. 41.

Jones, R.A.C. 1988. Seed-borne virus diseases in annual pasture legumes. Journal of Agriculture - Western Australia, Fourth Series 29, 58-61.

Jones, R.A.C. 1988. Seed-borne cucumber and alfalfa mosaic viruses in breeding and evaluation programmes of lupins and annual pasture legumes in Western Australia. Abstracts of the 11th Meeting of the International Working Group on Legume Viruses, Kurashiki, Japan. p. 6.

Jones, R.A.C. 1989. Losses caused by infection with cucumber and alfalfa mosaic viruses in legumes in Western Australia. Abstracts of 7th Meeting of Australasian Plant Pathology Society, Brisbane. p. 84.

Jones, R.A.C. 1990. Seed-borne virus diseases in the Western Australian lupin, subterranean clover and annual medic seed collections and breeding/evaluation programmes. In Proceedings of Workshop on Production, Maintenance and Exchange of Healthy Legume Germplasm, Melbourne. p. 31.

Jones, R.A.C. 1991. Losses in productivity of subterranean clover swards caused by sowing cucumber mosaic virus-infected seed. *Annals of Applied Biology* 119, 273-288.

Jones, R.A.C. 1992. Further studies on losses in productivity caused by infection of annual pasture legumes with three viruses. Australian Journal of Agricultural Research 43, 1229-1241.

Jones, R.A.C. 1993. Effects of three viruses on the productivity of annual pasture legume swards. In "Proceedings of XVII International Grasslands Conference; New Zealand. pp. 937-939.

Jones, R.A.C. 1995. Seed-borne virus diseases in alternative grain legumes. *Australian Grain, Western Focus* 5 (5), vi..

Jones, R.A.C. 1994. Infection of subterranean clover swards with bean yellow mosaic potyvirus: losses in herbage and seed yields and patterns of virus spread. Australian Journal of Agriculural Research

Jones, R.A.C. 1996. Virus diseases of Australian pastures. In Pasture and Forage Crop Pathology, pp. 288-307. Eds Chackraborty, S., Leath, K.T., Skipp, R.A., Pederson, G.A., Bray, R.A., Latch, G.C.M. and Nutter, F.W. American Society of Agronomy, Madison, Wisconsin, USA.

Jones R.A.C. 1999a. Bean yellow mosaic virus infection of alternative pasture legume species. In 1999 Cereals Update. (Eds C. Zaichou and N. Kerr). Rendezvous Observation City Hotel, Perth, 17-18 February 1999. Pages. 33-34.

Jones, R.A.C, 1999b. Bean yellow mosaic virus infection of alternative annual pasture legume species.In: Jones, R.A.C. (Ed.), Proceedings of the 15th Meeting of the International Working Group on legume Viruses. Centre for Legumes in Mediterranean Agriculture, Perth, Western Australia, p. 72.

Jones, R. A.C. 2000. Determining threshold levels for seed-borne virus infection in seedstocks. *Virus Research* 71, 171-183.

Jones, R. 2001. Widespread lucerne plantings pose disease risk to other crops.*Australian Grain* 11, 40-41.

Jones, R.A.C. 2004a. Using epidemiological information to develop effective integrated virus disease management strategies – a review. *Virus Research* 100, 5-30.

Jones, R.A.C. 2004b. Occurrence of virus infection in seed stocks and 3-year-old pastures of lucerne(*Medicago sativa)*. *Australian Journal of Agricultural Research* 55, 757-764.

Jones, R.A.C. 2006. Control of plant virus diseases. *Advances in Virus Research* 67, 205-244.

Jones, R.A.C., Coutts, B.A., Strickland, G.R., Kehoe, M.A., Severtson, D.L. 2008. The epidemiology of *Wheat streak mosaic virus* in Australia. Abstracts of 8th Australasian Plant Virology Workshop, Lake Okataina, Rotorua, New Zealand, November 19-22, p. 27.

Jones, R.A.C., Ferris, D.G. 1995. Insecticide control of AMV and insect pests in medics. *Australian Grain* (3), 36-38.

Jones, R.A.C., Ferris, D.G. 1995. Application of insecticides to limit spread of alfalfa mosaic virus in grazed annual medic swards. Proceedings of 6th International Plant Virus Epidemiology Symposium, Jerusalem, Israel, April 1995. p 7-8.

Jones, R. A. C. and Ferris, D.G. 1999. Alfalfa mosaic virus infection of grazed annual medic swards: suppressing spread using insecticides or admixture with grass, and impacts of infection on seed yields and stem coumestrol content. In Proceedings of 7th Australasian Conference on Grassland Invertebrate Ecology. Ed. J N Matthiessen. CSIRO: Perth, Western Australia, p. 296.

Jones R.A.C., Ferris D.G. 1999. Alfalfa mosaic virus infection of grazed annual medic swards: suppressing spread using insecticides or admixture with grass, and impacts of infection on seed yields and stem coumestrol content. In Proceedings of 15th Meeting of the International Working Group on Legume Viruses, Perth, Western Australia, August 15-17, 1999.

Jones, R.A.C. , Ferris, D.J. 2000. Suppressing spread of alfalfa mosaic virus in grazed legume pasture swards using insecticides and admixture with grass, and effects of insecticides on numbers of aphids and three other pasture pests. *Annals of Applied Biology* 137, 259-271.

Jones, R.A.C., Ferris, D.J. 2001. Virus infection stimulates phyto-oestrogen production in pasture legume plants growing in grazed swards. Annals of Applied Biology 138, 171-179.

Jones, R. A. C., Fosu-Nyarko J., Jones, M. G. K., and Dwyer, G I. 2001. Subterranean clover mottle virus. *AAB Descriptions of Plant Viruses*. No. 329. 8pp.

Jones, R.A.C., McKirdy, S.J. 1990. Seed-borne cucumber mosaic virus infection of subterranean clover in Western Australia. *Annals of Applied Biology* 116, 73-86.49.

Jones, R.A.C., McKirdy, S.J., Shivas, R.G. 1990. Occurrence of barley yellow dwarf virus in over- summering grasses and cereal crops in Western Australia. *Australasian Plant Pathology* 19, 90-96.

Jones, R.A.C., Nicholas, D.A. 1991. Alfalfa mosaic virus infection of grazed burr medic swards: seed infection, disease progress and effects on productivity. Abstracts of 8th Australasian Plant Pathology Society Conference, Sydney.

Jones, R.A.C., Nicholas, D.A. 1992. Studies on alfalfa mosaic virus infection of burr medic (*Medicago polymorpha)* swards: seed-borne infection, persistence, spread and effects on productivity. *Australian Journal of Agricultural Research* 43, 697-715.

Jones, R. A. C., Nicholas, D. A. 1997. Impact of an insidious virus disease in the legume component on the species balance within self-regenerating annual pasture. Proceedings of the 11th Biennial Australasian Plant Pathology Society Conference, Perth. p. 123.

Jones, R.A.C, Nicholas, D.A. 1998. Impact of an insidious virus disease in the legume component on the species balance within self-regenerating annual pasture. *Journal of Agricultural Science, Cambridge* 131, 155-17.

Jones, R.A.C., Latham, L.J. and Coutts, B.A. 2004. Devising integrated disease management tactics against plant viruses from 'generic' information on control measures. Agricultural Science, Australia 17, 10-18.

Jones, R.A.C. and Pathipanawat, W. 1989. Seed-borne alfalfa mosaic virus infecting annual medics (*Medicago* spp.) in Western Australia. Annals of Applied Biology 115: 263-277.

Jones, R.A.C., Ridsdill-Smith, T.J. 1996. IPM and plant resistance to pests and diseases of legumes in mediterranean climates: the CLIMA approach. In Proceedings of 'IPM- The Way Forward'. Co- operative Research Centres' Workshop, Avcare 1996 Annual Convention, Perth, Western Australia. pp. 1-12.

Kable, P. 1967. Reaction of some pasture legumes to a species of *Oidium* at Yanco, N.S.W. *Agricultural Gazette of New South Wales* 78, 611-12.

Kalla, R., Chu, P., Spangenberg, G. 2001. Molecular breeding of forage legumes for virus resistance. In "Molecular breeding of forage crops. Proceedings of the 2nd International Symposium, Molecular Breeding of Forage Crops, Lorne and Hamilton, Victoria, Australia, 19-24 November, 2000", G Spangenberg, ed. Kluwer Academic Publishers, Dordrecht. pp 219-237.

Karan M., Dale J. L., Bateson M. F., Harding R. M., Teakle D. S. 1994. Detection and characterization of pangola stunt Fijivirus from Australia using cloned cDNA probes. *Archives of Virology* 135, 397.

Kellock, A.W. 1971a Red-leaf virus - newly recognized virus disease of subterranean clover (*Trifolium subterraneum* L.) *Australian Journal of Agricultural Research* 22, 615-624.

Kellock, A.W. 1971b. Scorch disease can ruin sub.clover stands. *Journal of Agriculture, Victoria* 69, 328-9.

Kellock, A.W. 1972. A fungus that rots the roots of subterranean clover. *Journal of Agriculture, Victoria* 70, 112-113.

Kellock, A.W. 1975. A study of pathological and ecological factors causing root rot of subterranean clover (*Trifolium subterraneum* L.). Ph.D. Thesis, University of Melbourne, 202 pp.

Kellock, A.W., Stubbs, L.L. and Parbery, D.G. 1978. Seed-borne *Fusarium* species on subterranean clover and other pasture legumes. *Australian Journal of Agricultural Research* 29, 975-982.

Khair, G.T. 1981. List of plant parasitic nematodes of Australia. Canberra; Plant Quarantine, Department of Health, Commonwealth of Australia.

Kollmorgen, J.F. 1974. The pathogenicity of *Fusarium avenaceum* to wheat and legumes and its association with crop rotations. *Australian Journal of Experimental Agriculture and Animal Husbandry* 14, 572-576.

Kouame, C.N., Baltensperger, D.D., Prine, G.M. and Dunn, R.A. 1989. Screening subterranean clover (*Trifolium* spp.) germplasm for resistance to *Meloidogyne* species. *Journal of Nematology* 21,

Latham, L. J., McKirdy S. J., Jones, R. A. C. 1999. Cucumber mosaic virus in alternative cool season grain, annual pasture and forage legumes: susceptibility, sensitivity and seed transmission. In Proceedings of 15th Meeting of the International Working Group on Legume Viruses, Perth, Western Australia, August 15-17, 1999.

Latham, L.J., McKirdy S.J., Jones, R.A.C. 1999. Cucumber mosaic virus in alternative cool season grain, annual pasture and forage legumes: susceptibility, sensitivity and seed transmission. In Proceedings of 12th Biennial Australasian Plant Pathology Society Canference, Canberra, ACT, September, 1999.

Latham L.J., Jones, R A C. 1999. Alfalfa mosaic virus in alternative cool season grain, annual pasture and forage legumes: susceptibility, sensitivity and seed transmission. In Proceedings of 15th Meeting of the International Working Group on Legume Viruses, Perth Western Australia, August 15-17, 1999.

Latham, L.J., Jones, R.A.C. 2000. Alfalfa mosaic virus in alternative annual pasture and forage legumes.Eds Regan, K., White P. and Siddique K. Rendezvous Observation City Hotel, Perth, 16-17 February 2000. Pages?

Latham, L.J., Jones, R.A.C. 2001a. Alfalfa mosaic and pea seed-borne mosaic viruses in cool season crop, annual pasture, and forage legumes: susceptibility, sensitivity and seed transmission. *Australian Journal of Agricultural Research* 52, 771-790.

Latham, L.J., Jones, R.A.C. 2001b. Incidence of virus infection in experimental plots, commercial crops and seed stocks of cool season crop legumes. Australian Journal of Agricultural Research 52, 397-413.

Latham, L.J., Jones, R.A.C., and McKirdy, S.J. 2001. Cucumber mosaic cucumovirus infection of cool- season crop, annual pasture, and forage legumes: susceptibility, sensitivity and seed transmission. *Australian Journal of Agricultural Research* 52, 683-697.

Latter, D.H. 1953. Physiologic races of subterranean clover rust. *The Journal of the Australian Institute of Agricultural Science* 19, 248-50.

Li, H., Han, S., Nichols, P.G.H., Revell, C.K., Sivasithamparam, K., and Barbetti, M.J. 2009a. Responses of genotypes from species of *Trifolium, Ornithopus, Biserrula* and *Hedysarum* to a highly virulent race of *Phytophthora clandestina* and new sources of resistance. *Annals of Applied Biology* 155, 259-265.

Li, H., Han, S., Nichols, P.G.H., Revell, C.K., Sivasithamparam, K., and Barbetti, M.J. 2009b. Resistance to race 2 and cross-resistance to race 1 of *Kabatiella caulivora* in *Trifolium subterraneum* and *T. purpureum. Australasian Plant Pathology* 38, 284-287.

Loftus Hills, K. 1942. The reaction of varieties of *Trifolium subterraneum* to leaf rust (*Uromyces trifolii* (Hedw.) Lev.). *Journal of the Council for Scientific and Industrial Research* 15, 272-4.

Loftus Hills, K. 1944. The reaction of varieties of *Trifolium subterraneum* to attack by *Uromyces trifolii* as a heritable character. *Journal of the Council for Scientific and Industrial Research* 17, 74-8.

Ludbrook, W.V., Brockwell, J. and Riceman, D.S. 1953. Bare-patch disease and associated problems in subterranean clover pastures in South Australia. *Australian Journal of Agricultural Research* 4, 403-413.

Lynes, E.W., Teakle, D.S., Smith, P.F. 1981. Red clover necrotic mosaic virus isolated from *Trifolium repens* and *Medicago sativa* in Victoria. *Australasian Plant Pathology* 10, 6-7.

Ma, X Li, H., O'Rourke, T., Sivasithamparam, K. and Barbetti, M.J. 2008. Co-occurrence of an *Aphanomyces* sp. and *Phytopththora clandestina* in subterranean clover pastures in the high rainfall areas of the lower south-west of Western Australia. *Australasian Plant Pathology* 37, 74-78.

Ma, K., Kong, P., You, M.P., Li, H., Sivasithamparam, K., Barbetti, M.J. 2009. Molecular variation among isolates belonging to eight races of *Phytophthora clandestina*. *Australasian Plant Pathology* 38, 608-616.

Ma, XL, Li, H., Sivasithamparam, K., Barbetti, M.J. 2010. Infection processes and involvement of defense-related genes in the expression of resistance in cultivars of subterranean clover (*Trifolium subterraneum*) to *Phytophthora clandestina*. *Phytopathology* 100, 551-559.

Maughan, R.D. and Barbetti, M.J. (1983). Rhizoctonia root rot of white clover. *Australasian Plant Pathology* 12, 13-14.

McDonald, W., Nikandrow, A., Bishop, A., Lattimore, M., Gardner, P., Williams, R. and Hyson, L. 2003. Lucerne for Pasture and Fodder. NSW Agriculture Agfact P2.2.25, 3rd edn. Orange, NSW, Australia: NSW Agriculture.

McGee, D.C. and Kellock, A.W. 1974. Fusarium avenaceum - a seed-borne pathogen of subterranean clover roots. *Australian Journal of Agricultural Research* 25, 549-557.

McGechan J. 1980. *Yellows. Research Report* 11. NSW, Australia: BCRI (New South Wales Department of Agriculture, Biological and Chemical Research Institute).

McLean, G.D. 1983. Virus diseases of pasture legumes. Journal of the Western Australian Department of Agriculture No. 1, 12-13.

Mackie, J.M., Lloyd, D.L., Ryley, M.J. and Irwin, J.A.G. 1999. Fungal diseases of temperate annual pasture legumes in southern Queensland. *Australian Journal of Experimental Agriculture* 39, 699-707.

McKirdy, S.J., Jones, R.A.C. 1993. Perennial grasses as oversummering reservoirs for BYDV serotypes PAV, MAV, RPV and RMV in a region with a Mediterranean type climate. Abstracts of BYDV session, 6th International Congress of Plant Pathology, Montreal, Canada.

McKirdy, S.J., Jones R.A.C. 1993. Perennial grasses as oversummering reservoirs of BYDV serotypes MAV and RMV the South West of Western Australia. In Proceedings of 9th Biennial Conference, Australasian Plant Pathology Society, Hobart, Tasmania.

McKirdy, S.J., Coutts, B.A., Jones, R.A.C. 1994. Occurrence of bean yellow mosaic virus in subterranean clover pastures and perennial native legumes. *Australian Journal of Agricultural Research* 45, 183-194.

McKirdy, S.J., Jones, R.A.C. 1993. Occurrence of barley yellow dwarf virus serotypes MAV and RMV in over-summering grasses. *Australian Journal of Agricultural Research* 44, 1195-1209.

McKirdy, S.J., Jones, R.A.C. 1994a. Infection of alternative hosts associated with annual medics (*Medicago* spp.) by alfalfa mosaic virus and its persistence between growing seasons. *Australian Journal of Agricultural Research* 45, 1413-1426.

McKirdy, S.J., Jones R.A.C. 1994b. Infection of alternative hosts associated with narrow-leafed lupins (*Lupinus angustifolius*) and subterranean clover (*Trifolium subterraneum*) by cucumber mosaic virus and its persistence between growing seasons. *Australian Journal of Agricultural Research* 45, 1035-1049.

McKirdy, S.J., Jones, R.A.C. 1995a. Bean yellow mosaic potyvirus infection of alternative hosts associated with subterranean clover (*Trifolium subterraneum*) and narrow-leafed lupins (*Lupinus angustifolius*): field screening procedure, relative susceptibility/resistance rankings, seed transmission and persistence between growing seasons. *Australian Journal of Agricultural Research* 46, 135-152.

McKirdy, S.J., Jones, R.A.C. 1995b. Occurrence of alfalfa mosaic and subterranean clover red leaf viruses in legume pastures in Western Australia. *Australian Journal of Agricultural Research* 46,
763-774.

McKirdy, S.J., Jones, R.A.C. 1996. Use of imidacloprid and newer generation synthetic pyrethroids to control the spread of barley yellow dwarf luteovirus in cereals. *Plant Disease* 80, 895-901.

McKirdy, S.J., Jones, R.A.C. 1997a. Further studies on the incidence of virus infection in white clover pastures. *Australian Journal of Agricultural* 48, 31-37.

McKirdy, S.J., Jones, R.A.C. 1997b. Effect of sowing time on barley yellow dwarf virus infection in wheat:virus incidence and grain yield losses. *Australian Journal of Agricultural Research* 48, 199-206.

McKirdy S. J., Jones, R A C. 1998a. Bean yellow mosaic virus infection of annual pasture, forage and crop legumes in Australia. In Proceedings of the 7th International Congress of Plant Pathology (ICPP), Edinburgh,Scotland. 9-16, August, 1998, Poster No. 1.11.1.

McKirdy S.J., Jones R.A.C. 1999b. Contact transmission of viruses in pastures by grazing animals. In Proceedings of 15th Meeting of the International Working Group on Legume Viruses, Perth, Western Australia, August 15-17, 1999.

McKirdy, S.J., Jones, R.A.C., Barbetti, M.J. 1998a. Emerging disease problems in new pasture species. In Cereal Research and Industry Development in Western Australia, 1998. Updates, Observation City, February 1998 p. 54-55.

McKirdy, S. J., Jones, R. A. C., Sivasithamparam, K. 1997. Determining the effectiveness of grazing and trampling by livestock in transmitting white clover mosaic and subterraean clover mottle viruses. Proceedings of the 11th Biennial Australasian Plant Pathology Society Conference, Perth. p. 125.

McKirdy, S.J., Jones, R.A.C., Sivasithamparam, K. 1998b. Determining the effectiveness of grazing and trampling by livestock in transmitting white clover mosaic and subterranean clover mottle viruses. *Annals of Applied Biology* 132, 91-105.

McKirdy, S.J., Jones, R.A.C., Latham, L.J., Coutts, B.A. 1999. Bean yellow mosaic potyvirus in alternative cool season grain, annual pasture and forage legumes: susceptibility, sensitivity and seed transmission. In Proceedings of 15th Meeting of the International Working Group on Legume Viruses, Perth, Western Australia, August 15-17, 1999.

McKirdy, S.J., Jones, R.A.C., Latham, L.J. and Coutts, B.A. 2000. Bean yellow mosaic potyvirus infection of alternative annual pasture, forage and cool season crop legumes: susceptibility, sensitivity and seed transmission. *Australian Journal of Agricultural Research* 51, 325-345.

McLaughlin, M.R., Pederson, G.A., Evans, R.R., Ivy, R.L. 1992. Virus diseases and stand decline in a white clover pasture. *Plant Disease* 76,158-162.

McLean, G.D. 1983. Virus diseases of pasture legumes. *Journal of Agriculture, Western Australia.* 1, 12-3.

McLean G.D., Price, L.K., 1984. Virus, viroid, mycoplasma and rickettsial diseases of plants in Western Australia. Western Australian Department of Agriculture Technical Bulletin No. 68, 22p.

McLeish LJ, Berg GN, Hinch JM, Nambiar LV, Norton MR. 1997. Plant parasitic nematodes in white clover and soil from white clover pastures in Australia. *Australian Journal of Experimental Agriculture* 37, 75-82.

McMullen CL, Aldoud RA, Price TV, Flett SP, Clayton PG. 1997. The impact of root and stolon rots of white clover in Dairy pastures. In ‘Proceedings of the 11th biennial conference of Australasian Plant Pathology Society’. Perth, Australia. p. 141. Australasian Plant Pathology Society: Perth)

MacNish, G.C., Barbetti, M.J., Gillespie, D. and Hawley, K. 1976. Root rot of subterranean clover in Western Australia. *Journal of Agriculture, Western Australia* 17, 16-19.

Marcley, M.D. 1970. *Fusarium oxysporum* as a cause of lucerne decline in Western Australia. *Plant Disease Reporter* 54, 1061-1063.

Marks, G.C. and Mitchell, J.E. 1971a. Penetration and infection of alfalfa roots by *Phytophthora megasperma* and pathological anatomy of infected roots. *Canadian Journal of Botany* 49, 63-67.

Marks, G.C., and Mitchell, J.E. 1971b. Factors involved with the reaction of alfalfa to root rot caused by*Phytophthora megasperma*. *Phytopathology* 61, 510-514.

Maughan, R.D. and Barbetti, M.J. 1983. Rhizoctonia root rot of white clover. *Australasian Plant Pathology* 12, 13-14.

Mebalds, M.I. 1986. Effect of seed mycoflora on the development of disease in stands of *Medicago truncatula*. 21st International Seed Testing Association Congress, Brisbane, 10-19 July 1986. Preprint No. 43, 20p. (International Seed Testing Association: Secretariat, Zurich).

Mebalds, M.I. 1987. Mycoflora of *Medicago truncatula*, *M. rugosa* and *M. littoralis* seed produced in Victoria, Australia. *Seed Science and Technology* 15, 175-183.

Mekwatanakarn, P. 1985. Predisposition of virus infected white clover to fungal root rots. Preliminary Course Thesis, M.Sc. (Agriculture), University of Western Australia.

McGechan J, 1980. Yellows. Research Report 11. NSW, Australia: BCRI (New South Wales Department of Agriculture, Biological and Chemical Research Institute).

McLean LK, Price GD (1984) Virus, viroid, mycoplasma and rickettsial diseases of plants in Western Australia. Western Australian Department of Agriculture Technical Bulletin No. 68, 22p.

Moore, K.C. and Butler, B.J. 1979. Root rot resistant lucerne - a big leap forward. *Agricultural Gazette, New South Wales* 90, 36-67.Muller, W.J. Helms, K, Waterhouse, P.M. 1993. National survey of viruses in pastures of subterranean clover, 2. Statistical methodology for large scale quantitative ELISA. *Australian Journal of Agricultural Research* 44, 1863-1881.

Murray, G.M. and Davis, R.D. 1996. Fungal, bacterial and nematode diseases of Australian pastures. Pages 187-211 *In*: Pasture and Forage Crop Pathology. Eds S. Chakraborty, K.T.L. Leath, R.A. Skipp, G.A. Pederson, R.A. Bray, G.C.M. Latch and F.W. Nutter. ASA, CSSA, and SSSA, Madison, WI.

Murray, G.M. and Smith, A.M. 1970. *Cochliobolus sativus* causing a leaf blight disease of ryegrass (*Lolium* spp.) in New South Wales. *Search* 1, 79.

Musial, J.M., Aiten, K.S., Mackie, J.M. and Irwin, J.A.G. 2005. A genetic linkage map in auotetraploid lucerne adapted to northern Australia, and use of the map to identify

DNA markers linked to resistance to *Phytophthora medicaginis*. *Australian Journal of Agricultural Research* 56, 333-344.

Musgrave, D.R., Clifford, P.T.P. 1984. The effect of alfalfa mosaic virus on seed production in *Trifolium repens*. In 'Summary of research for the year ended 30 June 1984'. p. 14-15. (Plant Diseases Division, Department of Scientific and Industrial Research, New Zealand.)

Musgrave, D.R., Ebdon, S.C., Grose, T.A., Dawson, M., Ferguson, J.D. 1984. Identification of virus resistance in inter-specific crosses between *Trifolium repens*, *T. hybridium* and *T. ambiguum*. In 'Summary of research for the year ended 30 June 1984'. p. 14 (Plant Diseases Division,
Department of Scientific and Industrial Research, New Zealand).

Neal, J.S., Dennis, J., Scurrah, M., Bellotti, W.D., Slattery, J. and Hill, C.R. 1997a. Nutrition and disease control improve medic pastures. Page 68 *In*: Proceedings of the 11th Biennial Australasian Plant Pathology Conference, Perth, Australia, 29 September–2 October 1999.

Neal, J.S., Bellotti, B., Vanstone, B. and Graham, R. 1997b. Root lesion nematodes reduce annual medic growth. Australasian Plant Pathology Society. 11th Biennial Conference. Programme and Summaries. p.158. 29 September - 2 October. Rendezvous Observation City Hotel, Perth, Western Australia.

Neate, S.M. 1994. Soil and crop management practices that affect root diseases of cotton.
Pages 96-106
In: Management in sustainable farming systems. Eds C.E. Pankhurst, B.M. Doube, V.V.S.R. Gupta and P.R. Grace. CSIRO Australia, Melbourne, Australia.

Nicholas, D.A. 1980a. Larisa - a clover for very wet areas. Department of Agriculture, Western Australia, Farmnote 18/80.

Nicholas, D.A. 1980b. Esperance clover. Department of Agriculture, Western Australia,

Farmnote 61/80. Nicholas, D.A. 1980c. rikkala - an improved subterranean clover.

Department of Agriculture, Western Australia, Farmnote 85/80.

Nicholas, D.A. 1985a. Dalkeith and Junee subterranean clovers. Department of Agriculture, Western Australia, Farmnote 14/85.

Nicholas, D.A. 1985b. Green Range and Karridale subterranean clovers. Department of Agriculture, Western Australia, Farmnote 19/85.

Nicholas, D.A. 1980c. Trikkala - an improved subterranean clover. Department of Agriculture, Western Australia, Farmnote 85/80.

Nichols, P.G.H. 1992a. Goulburn subterranean clover. Department of Agriculture Western Australia, Farmnote 13/92.

Nichols, P.G.H. 1992b. Denmark subterranean clover. Department of Agriculture Western Australia, Farmnote 14/92.

Nichols, P.G.H. 1993. Gosse - a new subterranean clover for waterlogged soils. Department of
Agriculture Western Australia, Farmnote 92/93.

Nichols, P and Barbetti, M.J. 2005a. Coolamon – a productive, clover scorch-resistant subterranean clover for high rainfall mixed farming. Department of Agriculture Western Australia, Farmnote 4/2005.

Nichols, P and Barbetti, M.J. 2005b. Denmark - a persistent late flowering subterranean clover for high rainfall grazing. Department of Agriculture Western Australia, Farmnote 26/2005.

Nichols, P and Barbetti, M.J. 2005c. Gosse - a vigorous subterranean clover for high rainfall waterlogged soils. Department of Agriculture Western Australia, Farmnote 27/2005.

Nichols, P and Barbetti, M.J. 2005d. Goulburn - a persistent subterranean clover for high rainfall mixed farming. Department of Agriculture Western Australia, Farmnote 28/2005.

Nichols, P and Barbetti, M.J. 2005e. Napier - a highly productive subterranean clover for very high rainfall waterlogged soils. Department of Agriculture Western Australia, Farmnote 29/2005.

Nichols, P and Barbetti, M.J. 2005f. Riverina - a vigorous subterranean clover for medium to high rainfall waterlogged soils. Department of Agriculture Western Australia, Farmnote 30/2005.

Nichols, P and Barbetti, M.J. 2005g. Urana – a hardseeded subterranean clover with excellent winter vigour. Department of Agriculture Western Australia, Farmnote 31/2005.

Nichols, P and Barbetti, M.J 2005h. York - a persistent subterranean clover for medium-high rainfall mixed farming. Department of Agriculture Western Australia, Farmnote 32/2005.

Nichols, P.G.H., Barbetti, M.J. and You, M.P. 2008. Resistance to race 1 of *Kabatiella caulivora* in subterranean clover (*Trifolium subterraneum* L.) cultivars and breeding lines. *Australian Journal of Agricultural Research* 59, 561-566.

Nichols, P.G.H., Collins, W.J. and Barbetti, M.J. 1996. Registered cultivars of subterranean clover - their origin, identification, characteristics and use in Western Australia. Department of Agriculture, Western Australia, Bulletin No.4327. 61 pp.

Nichols, P.G.H., Evans, P.M., Craig, A.D., Sandral, G.A., Dear, B.S., Barbetti, M.J., Si, P., You, M.P. 2006a. Napier subterranean clover (*Trifolium subterraneum* L. var. *yanninicum* (Katz. Et Morley) Zohary and Heller). *Australian Journal of Experimental Agriculture* 46, 1109-1112.

Nichols, P.G.H., Sandral, G.A., Dear, B.S., de Koning, C.T., Lloyd, D.L., Evans, P.M., Craig, A.D., Barbetti, M.J., Si, P., You, M.P. 2006b. Urana subterranean clover (*Trifolium subterraneum* L. var. *subterraneum* (Katz. Et Morley) Zohary and Heller). *Australian Journal of Experimental Agriculture* 46, 1105-1107.

Nichols, P.G.H., Sandral, G.A., Dear, B.S., De Koning, C.T., Lloyd, D.L., Evans, P.M., Craig, A.D., Barbetti, M.J., Si, P., You, M.P. 2007a. Coolamon subterranean clover (*Trifolium subterraneum* L. var. subterraneum (Katz. et Morley) Zohary and Heller). *Australian Journal of Experimental Agriculture* 47, 223-225.

Nichols, P.G.H., Sandral, G.A., Dear, B.S., De Koning, C.T., Lloyd, D.L., Evans, P.M., Craig, A.D., Nutt, B.J., Barbetti, M.J., Si, P., You, M.P. 2007b. Izmir subterranean clover (*Trifolium subterraneum* L. var. *subterraneum*). *Australian Journal of Experimental Agriculture* 47, 226-229.

Nichols, P.G.H., Loi, A., Nutt, B.J., Evans, P.M., Craig, A.D., Pengelly, B.C., Lloyd, D.L., Dear, B.S., Revell, C.K., Nair, R.M., Ewing, M.A., Howieson, J.G., Auricht, G.A., Howie, J.H., Sandral, G.A., Carr, S.J., de Koning, C.T., Hackney, B.F., Crocker, G.J., Snowball, R., Hughes, S.J., Hall, E., Foster, K.J., Skinner, P.W., Barbetti, M.J. and You, M.P. 2007c. New annual and short-lived perennial pasture legumes for Australian agriculture - 15 years of revolution. *Field Crops Research* 104, 10-23.

Nichols, P.G.H., Snowball, R., D'Antuono, M.F. and Barbetti, M.J. 2010. Resistance to clover scorch (*Kabatiella caulivora*) among accessions of purple clover (*Trifolium purpureum*) and its relationship to eco-geography of collection sites. *Crop and Pasture Science* 61, 44-40.

Njeru, R., Ferris, D.G., Jones, R.A.C. and Jones, M.G.K. 1997. Studies on seed transmission of subterranean clover mottle virus and its detection in clover seed by ELISA and RT-PCR. *Australian Journal of Agricultural Research* 48, 343-350.

Njeru, R., Jones, R.A.C., Sivasithamparam, K. and Jones, M.G.K. 1995. Resistance to subterranean clover mottle virus in subterranean clover results from restricted cell-to-cell movement. *Australian Journal of Agricultural Research* 46, 633-643.

Norris, D.0. 1943. Pea mosaic on *Lupinus varius* L. and other species in Western Australia. Council for Scientific and Industrial Research Bulletin No. 170. 21 pp.

Norton, M.R., Johnstone, G.R. 1998. Occurrence of alfalfa mosaic, clover yellow vein, subterranean clover red leaf, and white clover mosaic viruses in white clover throughout Australia. *Australian Journal of Agricultural Research* 49, 723-728.

Ohki ST, Leps WT, Hiruki C (1986) Effects of alfalfa mosaic virus infection on factors associated with symbiotic N2 fixation in alfalfa. *Canadian Journal of Plant Pathology* 8, 277-281.

O'Loughlin, G.T. 1958. Clover stunt virus: a resistant variety of subterranean clover. Journal o the Department of Agriculture of Victoria 56, 385 - 381.

O'Neill, N.R. Bauchan, G.R. and Samac, D.A. 2003. Reactions in the annual *Medicago* spp. core germ plasm collection to *Phoma medicaginis*. *Plant Disease* 87, 557-562.

O'Rourke, T.A., Scanlon, T.T., Ryan, M.H., Wade, L.J., McKay, A.C., Riley, I.T., Li, H., Sivasithamparam, K. and Barbetti, M.J. 2009. Severity of root rot in mature subterranean clover and associated fungal pathogens in the wheatbelt of Western Australia. *Crop and Pasture Science* 60, 43-50.

O'Rourke, T.A., Ryan, M.H., Li, H., Ma, X., Sivasithamparam, K. Fatehi, J. and Barbetti, M.J. 2010.Taxonomic and pathogenic characteristics of a new species *Aphanomyces trifolii* causing root rot of subterranean clover (*Trifolium subterraneum*) in Western Australia. *Crop and Pasture Science* 61, 708–720.

Panetta, F.D., Ridsdill-Smith, T.J., Barbetti, M.J. and Jones, R.A.C. 1993. The ecology of weeds, invertebrate pests and diseases of Australian sheep pastures. In: Pests of Pastures; Weed, Invertebrate and Disease Pests of Australian Sheep Pastures (ed. E.S. Delfosse). pp. 87-114. Australian Wool Corporation Technical Publication; Commonwealth Scientific and Industrial Research Organisation : Melbourne.

Pathipanawat, W. Jones, R.A.C., Sivasithamparam, K. (1990). Alfalfa mosaic (AMV), cucumber mosaic (CMV) and bean yellow mosaic (BYMV) virus infection in annual medics (Medicago spp.). *Phytopathology* 80, 1082 (Abstr.).

Pathipanawat, W., Jones, R.A.C. and Sivasithamparam, K. 1994. An improved method for artificial hybridization in annual *Medicago* species. *Australian Journal of Agricultural Research* 45, 1329-1335.

Pathipanawat, W., Jones, R.A.C. and Sivasithamparam, K. 1995. Studies on seed and pollen transmission of alfalfa mosaic, cucumber mosaic and bean yellow mosaic viruses in cultivars and accessions of annual *Medicago* species. *Australian Journal of Agricultural Research* 46, 153-165.

Pathipanawat, W., Jones, R.A.C. and Sivasithamparam, K. 1996. Resistance to alfalfa mosaic virus in button medic (*Medicago orbicularis*). *Australian Journal of Agricultural Research* 47, 1157-1167.

Pathipanawat, W., Jones, R.A.C. and Sivasithamparam, K. 1997. Factors influencing transmission of alfalfa mosaic virus through seed of annual medics (*Medicago* spp.) and the genetic control of seed transmission rate. *Australian Journal of Agricultural Research* 48, 989-997.

Peak, J.W., Morley, F.H.W., Grylls, N.E. 1963. Resistance to subterranean clover stunt virus. *Australian Journal of Agricultural Research* 14, 639 - 645.

Peterson, S. 1954. Rust in subterranean clover. *Agricultural Gazette, New South Wales* 65, 597-602, 605.

Pilkington, L.J., Gibb, K.S., Gurr, G.M., Fletcher, M.J., NIkandrow, A., Elliott, E. van de., Ven, R.,Read, D.M.Y. 2002. First report of a phytoplasma associated with Australian lucerne yellows disease. *Plant Pathology* 51, 390

Pilkington, L.J., Gibb, K.S., Gurr, G.M., Fletcher, M.J., NIkandrow, A., Elliott, E. van de Ven, R., Read, D.M.Y. 2003. Detection and identification of a phytoplasma from lucerne with Australian lucerne yellows disease. *Plant Pathology* 52, 754-762.

Pilkington, L.J., Gurr, G.M., Fletcher, M.J., Nikandrow, A., Elliott, E. 1999. Occurrence and severity of lycerne yellows disease in Australian lucerne seed crops. *Australasian Plant Pathology* 28, 235-239.

Potter, L.R. 1993. The effects of white clover mosaic virus on vegetative growth and yield of on clones of S100 white clover. *Plant Pathology* 42, 797-805.

Pottinger, R.P., M.J. Barbetti, and T.J. Ridsdill-Smith. 1993. Invertebrate pests, plant pathogens and beneficial organisms of improved temperate pastures. Pages 909-918 *In*: Proceedings of the XVII International Grasslands Congress, Palmerston North, New Zealand, February 8-16, 1993.

Powell, N.T. 1971. Interactions between nematodes and fungi in disease complexes. *Annual Review ofPhytopathology* 9, 253-274.

Powles, B. 2001. Herbicide Resistance and World Grains. Eds. Powles SB, Shaner DL. CRC Press, Boca Raton London New York Washington, DC.

Price, L.K., McLean, G.D. 1982. Detecting alfalfa mosaic virus in sap. *Australasian Plant Pathology* 11 48 - 49.

Pung, S.H., Barbetti, M.J. and Sivasithamparam, K. 1988. Association of *Meloidogyne arenaria* with root rot of subterranean clover in Western Australia. *New Zealand Journal of Experimental Agriculture* 16, 91-96.

Pung, S.H., Barbetti, M.J. and Sivasithamparam, K. 1991a. The effect of aldicarb and benomyl on root- knot nematode and root rot in subterranean clover. *Australasian Plant Pathology* 20: 130-133.

Pung, S.H., Sivasithamparam, K. and Barbetti, M.J. 1991b. Effect of placement and sequence of inoculation with *Fusarium oxysporum* on its interaction with *Meloidogyne arenaria* on subterranean clover. *Journal of Phytopathology* 132, 273-286.

Pung, S.H., Barbetti, M.J. and Sivasithamparam, K. 1992a. Effect of soil environment on infection of subterranean clover by *Meloidogyne arenaria*. *Australian Journal of Agricultural Research* 43, 87-104.

Pung, S.H., Sivasithamparam, K. and Barbetti, M.J. 1992b. Interactions between *Fusarium oxysporum* and *Meloidogyne arenaria* as root pathogens of subterranean clover affected by soil sterilization and inoculum substrate. *New Zealand Journal of Agricultural Research* 35: 83-91.

Purss, G.S. 1959. Root rot of lucerne. *Queensland Agricultural Journal* 85, 767-770.

Purss, G.S. 1965. Diseases of lucerne. *Queensland Agricultural Journal* 91, 196-206.

Purwantara, A., Drenth, A., Flett, S.P., Guppy,W. and Keane, P. 2001. Diversity of *Phytophthora clandestina* isolated from subterranean clover in southern Australia: analysis of virulence and RAPD profiles. *European Journal of Plant Pathology* 107, 305-311.

Purwantara, A., Flett, S.P. and Keane, P.J. 1998. Variation in pathogenicity among isolates of *Phytophthora clandestina*. *Journal of Phytopathology* 146, 587-591.

Remah, A., Jones, A.T., Mitchell, M.J. 1986 Purification and properties of lucerne Australian symptomless virus, a new virus infecting lucerne in Australia. *Annals of Applied Biology* 109, 307-315.

Riley, I.T. and Kelly, S.J. 2002. Endoparasitic nematodes in cropping soils of Western Australia.
Australian Journal of Experimental Agriculture 42, 49-56.

Riley, I.T. and Wouts, W.M. 2001. *Pratylenchus* and *Radopholus* species in agricultural soils and native vegetation in southern Australia. *Transactions of the Royal Society of South Australia* 125, 147-153.

Rochow, W.F. 1970. Barley yellow dwarf virus. CMI/AAB Descritions of Plant Viruses No 32, 4pp.

Roget, D.K. 1995. Decline in root rot (*Rhizoctonia solani* AG-8) in wheat in a tillage and rotation experiment at Avon, South Australia. *Australian Journal of Experimental Agriculture* 35, 1009-1013.

Salam, M.U., Davidson, J.A., Thomas, G.J., Ford, R. and Jones R.A.C. et al (24 authors) 2011.Advances in winter pulse pathology research in Australia. *Australasian Plant Pathology* 40, 549-567.

Saqib, M., Wylie, S., Fosu-Nyarko, J.N., Smith T., Jones, M.G.K., Jones R..A.C. 2004. Molecular dissection of virus resistance using *Medicago truncatula*. In: Proceedings of 6th Australasian Plant Virology Workshop, Sea World Nara Resort, Gold Coast, Queensland, p.76.

Saqib, M., Fosu-Nyarko, J., Smith T., Wylie, S., Jones, R.A.C., Jones M.G K. 2005. Screening the core *Medicago truncatula* collection against four viruses: AMV, CMV, SCMoV, and BYMV. In Proceedings of 2nd Model Legume Workshop – Application to Crop and pasture improvement, Rottnest Island, WA, 5-8 April 2005. p. 25.

Saqib, M., Gajda, E., Jones, R.A.C., Jones, M.G.K. 2006. Identifying sources of resistance to three viruses within the core collection of the model legume Medicago truncatula. 7th Australasian Plant Virology Workshop. Rottnest Island, Perth, Western Australia. p 69.

Saqib, M., Ramsdale, R.A.., Jones, R.A.C, Jones, M.G.K. 2006. Identification of phytoplasma-associated disease in native and cultivated plants from Western Australia. 7th Australasian Plant Virology Workshop. Rottnest Island, Perth, Western Australia. P. 18.

Saqib,M., Jones, M.G.K. Jones, R.A.C. 2006. ‘Candidatus Phytoplasma australiense’ is associated withdiseases of red clover and paddy melon in south-west Australia. *Australasian Plant Pathology* 35,283-285.

Saqib, M., Ellwood, S., Jones, R.A.C., Jones M.G.K. 2008. Resistance to *Subterranean clover mottle virus* in *Medicago truncatula* and genetic mapping of a resistance locus. Abstracts of 8th Australasian Plant Virology Workshop, Lake Okataina, Rotorua, New Zealand, November 19-22, p. 35.

Saqib M., Ellwood, S.R., Jones R.A.C., and Jones M.G.K. 2009 Resistance to *Subterranean clover mottle virus* in *Medicago truncatula* and genetic mapping of a resistance locus. *Crop and Pasture Science* 60, 480-489.

Saqib, M., Gajda, B.E., Jones, M.G.K. and Jones R.A.C. 2011. Virus symptomatology in accessions of the *Medicago truncatula* core collection and identification of virus resistance phenotypes. *Crop and Pasture Science* 62, 686-700.

Svenson, K.G. and Venables, D.J. 1961. Detection of two legume viruses in Australia. Australian Journal of Experimental Agriculture and Husbandry 1,116-118.

Sward, R.J., Lister, R.M. 1988. The identity of barley yellow dwarf virus isolates in cereals and grasses from mainland Australia. *Australian Journal of Agricultural Research* 39, 375-384.

Sharma, H.C, Gill, B.S., Uyemoto, J.K. 1984. High levels of resistance in *Agropyron* species to barley yellow dwarf and wheat streak mosaic viruses. *Phytopathologisch Zeitshrift* 110, 143-147.

Sharma, S. 2001. Potential nematode threats associated with lucerne cultivation in Western Australia.Proceedings of the 2001 Crop Updates, Department of Agriculture Western Australia.

Shipton, W.A. 1967a. Diseases of clovers in Western Australia. *Journal of Agriculture, Western Australia* 8, 289-92.

Shipton, W.A. 1967b. Fungi associated with "purple patch" of subterranean clover in Western Australia.
Australian Journal of Science 30, 65.

Simpson, R.J., Richardson, A.E., Riley, I.T., McKay, A.C., McKay, S.F., Ballard, R.A., Ophel-Keller, K., Hartley, D., O’Rourke, T.A., Sivasithamparam, K., Li, H., Ryan, M.H. and Barbetti, M.J. 2011. Damage to roots of *Trifolium subterraneum* L. (subterranean clover), failure of seedlings to establish and the presence of root pathogens during autumn-winter. *Grass and Forage Science* (Online at DOI: 10.1111/j.1365-2494.2011.00822.x).

Sivasithamparam, K. 1993. Ecology of root-infecting pathogenic fungi in mediterranean environments.*Advances in Plant Pathology* 10, 245-279.

Slykhuis, J.T. 1959. A survey of virus diseases of grasses in Northern Europe. *Food and AgricultureOrganization Plant Protection Bulletin* 6, 129-134.

Slykhuis, J. T. 1962. An iniernational survey for virus diseases of grasses. *Food andAgricultureOrganization Plant Protection Bulletin* 10, 1-16.

Slykhuis, J.T., Paliwal, Y.C. 1972. Ryegrass mosaic virus. In CMI/AAB Descriptions of Plant Viruses'. No.86.

Smiley, R.W., Tayor, P.A., Clarke, R.G., Greenhalgh, F.C. and Trutmann, P. 1986. Simulated soil and plant management effects on root rots of subterranean clover. *Australian Journal of Agricultural Research* 37, 633-645.

Smith, H.C. 1957. Cereal yellow dwarf virus seen in Australia. *Commonwealth Phytiopathological News* 3, 10-11.

Smith, H.C. 1964: A survey of barley yellow dwarf virus in Australia in 1963. *New Zealand Journal ofAgricultural Research* 7, 239-247.

Stirling, G.R and Lodge, G.M. 2005. A survey of Australian temperate pastures in summer and winter rainfall zones: soil nematodes, chemical, and biochemical properties. *Australian Journal of Soil Research* 43, 887-904.

Stovold, G.E. 1971. Root rot research could check pasture decline. *Agricultural Gazette, New SouthWales* 82, 180.

Stovold, G.E. 1974a. Root rot caused by *Pythium irregulare* Buisman, an important factor in the decline of established subterranean clover pastures. *Australian Journal of Agricultural Research* 25, 537-48.

Stovold, G.E. 1974b. Root rot causes sub-clover decline. *Agricultural Gazette, New South Wales*, 85, 47. Stovold, G.E. 1976a. The relation of the severity of infection with *Kabatiella caulivora* to seed productionof subterranean clover and the seedborne nature of the pathogen in New South Wales.*Department of Agriculture, New South Wales, Plant Disease Survey* 1975-76, 21-24.

Stovold, G.E. 1976b. Lucerne root rot. Research Report 1976, Biological and Chemical Research Institute, Department of Agriculture, New South Wales, 6pp.

Stovold, G.E. 1983. Diseases of clover. Department of Agriculture, New South Wales, Agfact P2.AB.1. Stovold, G. and Rogers V. 1975. Root rot of lucerne. *Agricultural Gazette, New South Wales* 86, 48.

Stovold, G. and Wong, P. 1973. White clover decline on North Coast. *Agricultural Gazette, New South Wales* 84, 189.

Stuteville, D.L., Erwin, D.C. (Eds) 1990. Compendium of alfalfa diseases, 2nd Edn. 84p. (American Phytopathological Society: St Paul, MN).

Sulehuzzamen, M., Wilkins, P.W. 1984. Components of resistance to ryegrass mosaic virus in a clone of *Lolium perenne* and their strain specificity. *Euphytica* 33, 411-417.

Sward, R.J., Lister, R.M. 1988. The identity of barley yellow dwarf virus isolates in cereals and grasses from mainland Australia. *Australian Journal of Agricultural Research* 39, 375-384. *Annals of Applied Biology 120,* Supplement.

Tan, D., Flematti, G.R., Ghisalberti, E.L., Sivasithamparam, K., Chakraborty, S., Obanor, F. and Barbetti, M.J. 2011. Mycotoxins produced by *Fusarium* species associated with annual legume pastures and sheep feed refusal disorders in Western Australia. *Mycotoxin Research* 27, 123-135.

Tan, D., Flematti, G.R., Ghisalberti, E.L., Sivasithamparam, K. and Barbetti, M.J. 2011. Toxigenicity of enniatins from Western Australian *Fusarium* species to brine shrimp (*Artemia franciscana*). *Toxicon* 57, 817-825.

Taylor, N.L., Gibson, P.B., Knight, W.E. 1977. Genetic variability and germplasm resources in the true clovers. *Crop Science* 17, 632-634.

Taylor, N.L., Ghabrial, S.A. 1986. Breeding forage legumes for resistance to viruses. In: Viruses infecting forage legumes,Vol III, eds Edwardson,J.R., Christae, R.G. pp. 609-623. University of Florida: Gainsville.

Taylor, P.A. 1984). An unusual *Phytophthora* associated with root rot of subterranean clover.*Plant Disease* 68, 450.

Taylor, P.A., Barbetti, M.J. and Wong, D.H. 1985a. Occurrence of *Phytophthora clandestina* in Western Australia. *Plant Protection Quarterly* 1, 57-58.

Taylor, P.A., Clarke, P.G., Kelly, K. and Smiley, R.W. 1985b. Root rot of irrigated subterranean clover in northern Victoria : its significance and prospects for control. Pages 271-273 *In*: Ecology and Management of Soil-borne Plant Pathogens. Eds. C.A. Parker, W.J. Moore, P.T.W. Wong, A.D. Rovira and J.F. Kollmorgen. St Paul, U.S.A.; American Phytopathological Society.

Taylor, P.A. and Greenhalgh, F.C. 1987. Significance, causes and control of root rots of subterranean clover. Pages 249-251 *In:* Temperate Pastures: their Production, Use and Management. Eds JL Wheeler, CJ Pearson, and GE Robards. Australian Wool Corporation/Commonwealth Scientific and Industrial Research Organisation: East Melbourne.

Taylor, P.A., Pascoe, I.G. and Greenhalgh, F.C. 1985c. *Phytophthora clandestina* sp. nov. in roots of subterranean clover. *Mycotaxon* 22, 77-85.

Taylor, S.P., Hollaway, G.J. and Hunt, C.H. 2000. Effect of field crops on population densities of *Pratylenchus neglectus* and *P. thornei* in south eastern Australia; Part 1: *P. neglectus*. *Journal of Nematology* 32, 591-599.

Teakle, D.S., Hicks, S., Harding, R.M., Greber, R.S., Milne, R.G. 1988. Pangola stunt virus infecting pangola grass and summer grass in Australia. *Australian Journal of Agricultural Research* 39, 1075-1083.

Thackray, D.J., Jones, R.A.C., 1999. Role played by nearby pastures in epidemics of aphid-borne virus diseases in crops in regions with Mediterranean-type climates. In: Jones, R.A.C. (Ed.), Proceedings of the 15th Meeting of the International Working Group on Legume Viruses, Centre for legumes in Mediterranean Agriculture, Fremantle, Western Australia, p. 51

Thackray, D.J., Jones, R.A.C. 1999. Roles of nearby pastures in development of epidemics of aphid- borne virus diseases in crops in regions with Mediterranean-type climates In Proceedings of 7th Australasian Conference on Grassland Invertebrate Ecology. Ed. J N Matthiessen. CSIRO: Perth, Western Australia, p. 298.

Teakle, D.S. 1956. A lucerne root rot caused by *Pythium myriotylum*. *The Queensland Journal of Agricultural Science* 13, 241-243.

Teakle, D.S, Grylls, N.E. 1973. Four strains of sugar-cane mosaic virus infecting cereals and other grasses in Australia. *Australian Journal of Agricultural Research* 24, 465-477.

Teakle, Moore, George & Byth, *Aust. J. agric. Res.* **21**: 549, 1970.

Teakle, D.S, Hicks, S, . M. Harding, R.M., Greber R., Milne, R.G. 1988. Pangola stunt virus infecting pangola grass and summer Grass in Australia. *Australian Journal of Agricultural Research* 39, 1075-1083.

Thongbai, P., Hannam, R.J., Graham, R.D. and Webb, M.J. 1993. Interactions between zinc nutritional status of cereals and Rhizoctonia root rot severity. 1. Field observations. *Plant and Soil* 153, 207-214.

Tivoli, B., Baranger, A., Sivasithamparam, K. and Barbetti, M.J. 2006. Annual *Medicago* - from a model crop challenged by a spectrum of necrotrophic pathogens to a model plant to explore the nature of disease resistance. *Annals of Botany* 98, 1117-1128.

Tu, J.C. Holmes, T.M. 1980. Effect of alfalfa mosaic virus infection on nodulation, forage yield, forage protein, and overwintering of alfalfa. *Phytopathologische Zeitschrift* 97, 1-9.

Turner, N.C. and Asseng, S. 2005. Productivity, sustainability, and rainfall-use efficiency in Australian rainfed Mediterranean agricultural systems. *Australian Journal of Agricultural Research* 56, 1123-1136.

Valder, P.G. 1954. Diseases of clovers in New South Wales. *Agricultural Gazette of New South Wales* 65, 465-71.

Van Leur, J.A.G., Kumari, S.G. 2011. A survey of lucerne in northern New South Wales for viruses of importance to the winter legume industry. *Australasian Plant Pathology* 40, 180-186.

Vanstone, V.A. and Russ, M.H. 2001. Ability of weeds to host the root lesion nematodes *Pratylenchus neglectus* and *P. thornei*. I. Grass weeds. *Australasian Plant Pathology* 30, 245-250.

Walker, J. 1956a. Further clover diseases in New South Wales. *Agricultural Gazette of New South Wales* 67, 353-357.

Walker, J. 1956b. The reactions of subterranean clover varieties to scorch caused by *Kabatiella caulivora* (Kirchn.) Karak. *The Journal of Australian Institute of Agricultural Science* 22, 288-290.

Walker, J. 1965-66. Rusts of *Medicago* spp. in Australia and New Zealand. *Australian Journal of Science* 28, 168.

Walsh, M.J. Delaney, R.H. Groose, R.W. and Krall, J.M. 2001. Performance of annual medic species (*Medicago* spp.) in Southeastern Wyoming. *Agronomy Journal* 93, 1249-1256.

Waterhouse, W.L. 1951. Australian rust studies. VIII. *Puccinia graminis* lolii, an undescribed rust of *Lolium* spp. and other grasses in Australia. *Proceedings of the Linnean Society of New South Wales* 76, 57-64.

Waterhouse PM and Helms K 1985. *Metalolophium dirhodum* (Walker): a newly arrived vector of barley yellow dwarf virus in Australia. *Australasian Plant Pathology* 14, 64-66.

White, N.H., Cass-Smith, W.P. and Harvey, H.L. 1956. Wart disease of subterranean clover. *The Journal of the Australian Institute of Agricultural Science* 22, 204-5.

Wilhelm, N.S., Graham, R.D. and Rovira, A.D. 1990. Control of Mn status and infection rate by genotype of both host and pathogen in wheat take-all interaction. *Plant and Soil* 123, 267-275.

Williams BL, Pascoe IG. 1994. *Phytophthora megasperma*, a pathogen of *Trifolium repens* in irrigated pastures of northern Victoria. *Australasian Plant Pathology* 23, 88–96.

Wade, G.C. 1957. Diseases of pasture plants in Tasmania. *Tasmanian Journal of Agriculture* 28, 64 - 69.

White, N.H. 1945. Plant Disease Survey of Tasmania for the three year period 1943, 1944, 1945. Hobart: Tasmanian Department of Agriculture Monograph. 30 pp.

Wilkins, P.W. 1987. Transfer of polygenic resistance to ryegrass mosaic virus from perennial to Italian ryegrass by backcrossing. *Annals of Applied Biology* 111, 409-413.

Wilkins, P.W., Catherall, P.L. 1977. Variation in reaction to barley yellow dwarf virus in ryegrass and its inheritance. *Annals of Applied Biology* 85, 257-263.

Wong, D.H. 1986. Fungi associated with root rot of subterranean clover in south-west of Western Australia. PhD Thesis, University of Western Australia.

Wong, D.H., Sivasithamparam, K. 1985. *Rhizoctonia* spp. associated with root rots of subterranean clover in Western Australia. *Transactions of the British Mycological Society* 85, 21–27.

Wong, D.H., Barbetti, M.J. and Sivasithamparam, K. 1984. Effects of soil temperature and moisture on the pathogenicity of fungi associated with root rot of subterranean clover. *Australian Journal of Agricultural Research* 35, 675-684.

Wong, D.H., Barbetti, M.J. and Sivasithamparam, K. 1985a. Pathogenicity of *Rhizoctonia* spp. associated with root rots of subterranean clover. *Transactions of the British Mycological Society* 85, 156-158.

Wong, D.H., Barbetti, M.J. and Sivasithamparam, K. 1985b. Fungi associated with root rots of subterranean clover in Western Australia. *Australian Journal of Experimental Agriculture* 25, 574-579.

Wong, D.H., D'Antuono, M.F., Barbetti, M.J. and Sivasithamparam, K. 1986a. Inter-relationship between shoot weight, severity of root rot and survival rate of subterranean clover inoculated with certain pathogenic fungi. *Plant and Soil* 96, 141-143.

Wong, D.H., Barbetti, M.J. and Sivasithamparam, K. 1986b. Behaviour of *Phytophthora clandestina* propagules at a field site in Western Australia. *Australian Journal of Soil Research* 24, 483-491.

Wong, D.H., Sivasithamparam, K. and Barbetti, M.J. 1986c. Influence of soil temperature, moisture and other fungal root pathogens on the pathogenicity of *Phytophthora clandestina* to subterranean clover. *Transactions of the British Mycological Society* 86, 479-482.

Wong, D.H., Sivasithamparam, K. and Barbetti, M.J. 1986d. Soil behaviour of *Phytophthora clandestina*. *Journal of Phytopathology* 126: 67-73.

Wong, D.H., Sivasithamparam, K. and Barbetti, M.J. 1986e. Influence of environmental factors on the growth and survival of *Phytophthora clandestina*. *Canadian Journal of Microbiology* 32, 553-556.

Wong, D.H., Sivasithamparam, K. and Barbetti, M.J. 1986f. Pathogenicity of *Fusarium oxysporum* isolates associated with root rot of subterranean clover in Western Australia. *Phytophylactica* 18, 133-135.

Wong, P.T.W. 1975. Kikuyu yellows - A disease caused by an undescribed Phycomycete. *Plant Disease Reporter* 59, 800-801

Wright, D. and Burgess, N. 2002. Fungal diseases present in Western Australian lucerne crops. Proceedings of the 2002 GRDC Annual Crop Updates, Western Australia.

Wright, D. and Jones, R. 2003. Disease survey in three-year-old lucerne pastures. *Ground Cover* 43, 5

Wroth, J.M. and Jones, R.A.C. 1991. Virus diseases of subterranean clover. Farmnote, Western Australian Department of Agriculture No. 68/91.

Wroth, J.M. and Jones, R.A.C. 1992a. Subterranean clover mottle virus infection of subterranean clover: widespread occurrence in pastures and effects on productivity. *Australian Journal of Agricultural Research* 43, 1597-1608.

Wroth, J.M. and Jones, R.A.C. 1992b. Subterranean clover mottle sobemovirus: its host range, resistance in subterranean clover and transmission through seed and by grazing animals. *Annals of Applied Biology* 121, 329-343.

Wroth, J.M., Dilworth, M.J. and Jones, R.A.C. 1993. Impaired nodule function in *Medicago polymorpha* L. infected with alfalfa mosaic virus. *New Phytologist* 124, 243-250.

You, M. P., Sivasithamparam, K., Riley, I.T. and Barbetti M.J. 1999. Relationship of rainfall, cultural practices, soil and plant nutrients and seedling survival with root disease and parasitic nematode numbers in annual *Medicago* spp. pastures. *Australian Journal of Agricultural Research* 50, 977-983.

You, M. P., Sivasithamparam, K., Riley, I.T. and Barbetti M.J. 2000. The occurrence of root infecting fungi and parasitic nematodes in annual *Medicago* spp. in Western Australian pastures. *Australian Journal of Agricultural Research* 51, 435-444.

You, M., Barbetti, M.J. and Nichols, P.G.H. 2005a. New *Trifolium subterraneum* genotypes identified with combined resistance to race 2 of *Kabatiella caulivora* and cross-resistance to fungal root rot pathogens. *Australian Journal of Agricultural Research* 56, 1111-1114.

You, M.P., Barbetti, M.J. and Nichols, P.G.H. 2005b. New sources of resistance in *Trifolium subterraneum* L. to root rot caused by two races of *Phytophthora clandestina* Taylor, Pascoe and Greenhalgh. *Australian Journal of Agricultural Research.* 56, 271-277.

You, M., Barbetti, M.J. and Sivasithamparam, K. 2005c. Characterization of *Phytophthora clandestina* races on *Trifolium subterraneum* in Western Australia. *European Journal of Plant Pathology* 113, 267-274.

You, M., Barbetti, M.J. and Nichols, P.G.H. 2005d. New sources of resistance identified in *Trifolium subterraneum* breeding lines and cultivars to root rot caused by *Fusarium avenaceum* and *Pythium irregulare* and their relationship to seedling survival. *Australasian Plant Pathology* 34, 237-244.

You, M., Barbetti, M.J., Nichols, P.G.H. 2005e. New *Trifolium subterraneum* genotypes identified with combined resistance to race 2 of *Kabatiella caulivora* and cross-resistance to fungal root rot pathogens. *Australian Journal of Agricultural Research* 56, 1111-1114.

You, M., Barbetti, M.J. and Sivasithamparam, K. 2006. Occurrence of *Phytophthora clandestina* races across rainfall zones in south west Western Australia. *Australasian Plant Pathology* 35, 85-87.

You, M.P., Lancaster, B., Sivasithamparam, K. and Barbetti, M.J. 2008. Cross-pathogenicity of *Rhizoctonia solani* strains on pasture legumes in pasture-crop rotations. *Plant and Soil* 302, 203-211.

You, M.P., Yang, H.A., Sivasithamparam, K., and Barbetti, M.J. 2009. A new leaf blight disease of *Trifolium dasyurum* caused by *Botrytis fabae*. *European Journal of Plant Pathology* 123, 99-103.

Zahid M.I., Gurr, G.M., Nikandrow, A., Hodda, M., Fulkerson, W.J., Nicol, H.I. 2001a. Survey of fungi and nematodes associated with root and stolon diseases of white clover in the subtropical dairy region of Australia. *Australian Journal of Experimental Agriculture* 41, 1133–1142.

Zahid, M.I., Gurr, G.M., Nikandrow, A., Fulkerson, W.J., Nicol, H.I. 2001b. Pathogenicity of root and stolon-colonising fungi to white clover. *Australian Journal of Experimental Agriculture* 41, 763–771.

Zahid, M.I., Nobbs, J., Gurr, G.M., Hodda, M., Nikandrow, A., Fulkerson, W.J., Nicol, H.I. 2001c. Effect of the clover root-knot nematode (*Meloidogyne trifoliophila*) on growth of white clover. *Australasian Nematology* 3, 437–446.

Zahid, M.I., Nobbs, J, Stanton, J.M., Gurr, G.M., Hodda, M., Nikandrow, A, Fulkerson, W.J. 2000. First record of *Meloidogyne trifoliophila* in Australia. *Australasian Plant Pathology* 29, 280.

Zahid, M.I., Gurr, G.M., Nikandrow, A., Fulkerson, W.J., Nicol, H. 2002. Effect of cover crops and cultural treatments on plant parasitic nematode density, fungal root disease severity and yield in white clover. *Australian Journal of Experimental Agriculture* 42, 165–171.