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## **Role and Impact of Diseases caused by Soil– Borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems**

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# Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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## Abstract

The role and impact of diseases caused by soil-borne plant pathogens in reducing productivity in southern Australian pasture systems urgently needs defining. As a consequence, this report evaluates the research and extension articles published on diseases caused by soil-borne fungal and nematode pathogens of annual and perennial forage legume and grass pastures which are sown in south west, southern, and south eastern Australia and highlights those currently believed to adversely affect the growth and/or production of the affected plant species. It also includes some of the animal toxicoses from toxin producing soil-borne fungi which in their own right may or may not cause disease of the host species. A number of nematode and necrotrophic soil-borne pathogens have been associated with significant productivity decline and pose a serious threat to one or more annual or perennial forage legume or grass species to the extent that they require reseeded. At the current state of investigations the most important fungi associated with root disease in the most widely grown pasture legume, subterranean clover, appear to be, in order of importance, *Phytophthora clandestina*, *Pythium irregulare*, *Aphanomyces eutichies*, *Rhizoctonia species* and *F. avenaceum*. This report highlights the areas needing to be addressed if losses caused by soil-borne plant pathogens are to be reduced.

# Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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## Executive Summary

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

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

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

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

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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

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

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

There are a number of issues that, if addressed, should lead to improvement in the assessment of soil-borne fungal and nematode pathogen-induced losses occurring across southern Australia. It is desirable that the level and impact of individual soil-borne fungal and nematode pathogens and their complexes be defined throughout as much as possible of the geographic range of the pasture species they infect, within and between years, and include periods when feed is limiting. Herbage and seed yield losses should be assessed in grazed monoculture swards or, wherever possible, in mixed species swards or grazed commercial pastures, along with pathogen-induced changes in botanical composition, numbers of plants regenerating (especially for annual pastures), persistence after the first year, rotational effects (such as nitrogen availability) and factors affecting feed quality (such as phyto-oestrogens and mycotoxins). There would be significant benefit from improvement

## **Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems**

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of economic evaluation packages or models, from making them more user-friendly and, in particular, from employing them more widely. There is a clear need for improved extension to achieve a wider appreciation of the need for the management of soil-borne pathogens and the benefits derived from it. Given the labour-intensive and long term nature of field work involved with diseases in pastures, the tendency of funding to be targeted at increasingly short term minimal expenditure projects needs to be addressed if the necessary studies, targeting issues such as soil-borne pathogen-induced yield loss, are to occur.

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

This report covers research and extension articles published on diseases caused by soil-borne fungal and nematode pathogens of annual and perennial forage legume and grass pastures which are sown in south west, southern, and south eastern Australia and highlights those currently believed to adversely affect the growth and/or production of the affected plant species. It also includes some of the animal toxicoses from toxin producing soil-borne fungi which in their own right may or may not cause disease of the host species. This report in general does not cover soil-borne fungi and nematodes that are simply listed in records of fungal and nematode pathogens occurring on one or more hosts among the pasture species.

## **Contents**

<b>1</b>	<b>Introduction.....</b>	<b>9</b>
1.1	Role of Diseases in the Decline of Pasture and Forage Legumes	9
1.2	Pasture and Forage Diseases – Challenges .....	9
1.3	Necrotrophic Fungal Root Pathogens.....	10
<b>2</b>	<b>Soil-borne Diseases of Clovers.....</b>	<b>11</b>
2.1	Introduction .....	11
2.2	Symptoms.....	11
2.3	Impact.....	12
2.4	Environmental Influences.....	19
2.5	Seed-borne Fungal Pathogens.....	21
2.6	Soil-borne Nematodes .....	22
2.7	Soil-borne Pathogen Interactions with Viruses .....	23
2.8	Disease Management Using Chemicals .....	24
2.9	Disease Management Using Cultural Practices.....	25
2.10	Disease Management Using Host Resistance .....	26
<b>3</b>	<b>Soil-borne Diseases of Annual Medics.....</b>	<b>29</b>
3.1	Impact of Medic Root Diseases.....	29
3.2	Necrotrophic Fungal Foliar and Root Pathogens.....	30
3.2.1	Pathogen survival between seasons .....	30
3.2.2	Impact of necrotrophic pathogens causing root and/or foliar(e.g., crown and stem) diseases .....	31
3.2.3	Importance of necrotrophic pathogens from their side effects.....	31
3.2.4	Seed and burr health.....	32
3.3	<b>Soil-borne Diseases – Affect Critical Functions of Annual Medic Roots</b>	<b>32</b>
3.4	<b>Plant Parasitic Nematodes in Annual Medics .....</b>	<b>33</b>
3.5	<b>Host Resistance .....</b>	<b>34</b>
3.5.1	Individual sources of host resistance.....	35
3.6	<b>Cultural Control Strategies.....</b>	<b>36</b>
3.6.1	Grazing.....	36
3.6.2	Tillage and burning.....	36
3.6.3	Nutrition.....	37
3.6.4	Rotations .....	37
3.7	<b>Fungicidal Control Strategies .....</b>	<b>38</b>

# Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

---

3.8	Integrated Disease Management.....	38
3.9	Conclusion.....	38
<b>4</b>	<b>Soil-borne Diseases of Lucerne and Other New Legumes .....</b>	<b>39</b>
4.1	Introduction .....	39
4.2	Soil-borne Pathogens and their Impact.....	40
<b>5</b>	<b>Soil-borne Diseases of Grasses .....</b>	<b>44</b>
<b>6</b>	<b>Need to Improve Assessment of the Impacts of Soil-borne Diseases.....</b>	<b>46</b>
6.1	Introduction .....	46
6.2	Assessing Costs and Benefits of Controlling Disease-induced Losses	46
6.3	Where to Next in Relation to Improving Assessment of the Impacts of Soil-borne Diseases.....	48
<b>7</b>	<b>Interaction of Nutrition and Soil-borne Diseases</b>	<b>48</b>
7.1	Introduction .....	48
7.2	Nutrient Effects on ‘Pathogen Suppression’ .....	50
7.2.1	Saprophytic survival: dormancy .....	50
7.2.2	Saprophytic survival: growth on substrate – on crop residues for both legumes and grasses.....	51
7.2.3	Saprophytic growth in soil .....	51
7.2.4	Saprophytic colonization .....	52
7.3	Nutrient Effects on ‘Disease Suppression’ .....	53
7.3.1	Direct effects on host: enhancement of resistance.....	53
<b>8</b>	<b>Implications for Industry.....</b>	<b>54</b>
8.1	Soil-borne Pathogens – Lack of Key Information on Impacts..	55
8.2	Pasture Grasses.....	58
8.3	Nutrition Soil-borne Pathogen Interactions .....	58
<b>9</b>	<b>Recommendations for Future Research .....</b>	<b>58</b>
9.1	Diseases of New Legumes .....	58
9.2	Nutrients and IPM.....	59
9.3	Pathogen Complexes.....	59
9.4	Loss Assessments.....	59
9.5	Mycotoxins and Quality .....	59
9.6	Host Resistance in Annual Medics and Grasses.....	60

**Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing  
Productivity in Southern Australian Pasture Systems**

---

<b>9.7</b>	<b>Training .....</b>	<b>60</b>
<b>10</b>	<b>Bibliography .....</b>	<b>61</b>
<b>11</b>	<b>Summary Report for Researchers, Advisors and Producers .....</b>	<b>89</b>
<b>11.1</b>	<b>Soil-borne Disease Impacts .....</b>	<b>89</b>
<b>11.2</b>	<b>Important Soil-borne Pathogens.....</b>	<b>90</b>
<b>11.3</b>	<b>Disease Management - Options .....</b>	<b>91</b>



## **1 Introduction**

### **1.1 Role of Diseases in the Decline of Pasture and Forage Legumes**

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Farmers have difficulty in the recognition of certain if not most diseases in pastures, especially where they are not based on pure species and occur in mixed swards often with a significant component of weeds. This problem is more so in relation to root diseases rather than for foliar diseases. Forage legume crops and pastures consist of one or more of a range of annual or perennial plant species (e.g., annual medics, clovers, lotus, lucerne, stylo and an array of annual and perennial grasses). Over 400 fungal, bacterial, viral, mycoplasma and nematode diseases known to affect the productivity of pastures (Haggar *et al.*, 1984) and fungi, bacteria and viruses are all known to play a role in affecting pasture productivity in Australia (Murray and Davis, 1996). For some annual pasture legume species such as subterranean clover they have been extensively reviewed previously in relation to both foliar (Barbetti and Sivasithamparam, 1986) and root (Barbetti *et al.*, 1986) diseases. While each pasture species has individual diseases, some are common to a number of species, especially across annual pasture and forage legumes. Pathogens by definition cause losses that can adversely affect animal feed availability. Such losses can adversely affect industries such as wool, meat, dairy or grain production, and these losses have been reviewed to at least some extent previously, both in Australia (e.g., Johnstone and Barbetti, 1987; Barbetti *et al.*, 1996) and, more extensively, elsewhere (e.g., Graham *et al.*, 1979; Haggar *et al.*, 1984; Barnett and Diachun, 1985; Raynal *et al.*, 1989; Edwardson and Christie, 1986; Braverman *et al.*, 1986; Cook and Yeates, 1993; Lenné, 1994a,b). However, such reviews generally give limited or no coverage of annual medics or grasses and totally fail to address the situation of the immediate past 10-15 years or more.

This report firstly, outlines the major and/or most widespread diseases caused by necrotrophic fungal pathogens and plant parasitic nematodes; secondly, defines the role of necrotrophic fungal pathogens and plant parasitic nematodes in the loss of productivity of annual legume-based pastures; and, thirdly, investigates the full spectrum of control options for management. For this report, we based our definition of “necrotroph” on that of Agrios (2004), to mean an organism which has at least one part of their life cycle on dead host/tissue and which can grow on artificial nutrient media.

### **1.2 Pasture and Forage Diseases – Challenges**

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One of the unique challenges in dealing with pasture and forage legume diseases is that much of the published information on these diseases comes from experiments that are inadequate or not representative of the natural grazed pastures containing a mixture of plant species (Barbetti *et al.*, 1996). In part, this is due to the relative low unit value of many pasture and forage species, which discourages intensive investigation of both losses and of investigations that lead to better understanding of disease epidemiology and subsequent improved disease management strategies. In the absence of such intensive investigations it is difficult to make rational disease management decisions or to make accurate economic assessments in relation to pasture and forage diseases.

In recent years, there has been growing emphasis on improving productivity of annual pasture legumes by intensifying grazing and feed-base utilisation systems around the world (Barbetti *et al.*,

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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1996). Intensive grazing management often leads to increases in disease-induced losses in feed as production levels come closer to the limit of both pasture and individual cultivar potentials. This is particularly noticeable in areas where the utilisation of annual legume monocultures or near-monocultures in short leys is increasing, for while they often enhance yield potential, they do not provide the buffering benefits that result from having other genera present and therefore can exacerbate losses from diseases.

While losses in annual *Trifolium* spp. such as subterranean clover (Barbetti, 1987c; Pratt, 1989; Barbetti and Nichols, 1991a; Barbetti, 1996) and perennial *Medicago* species (Berkenkamp, 1971; Basu, 1976; Broscious *et al.*, 1987; Campbell and Guthrie, 1990) have been generally well quantified, those occurring in annual medics or grasses have not been well quantified. This is despite reports of declining medic production (Bellotti and Kerby, 1993). Losses on annual pasture and forage legumes caused by necrotrophic pathogens can be direct or indirect. Direct losses include diminished plant growth (*i.e.*, decreased herbage), nutritional value (*e.g.*, protein, individual amino acid and water soluble carbohydrate content, and dry matter digestibility), palatability, seed set and seed viability, and increased toxin production (*e.g.*, phyto-oestrogens, tannins, phenols, mycotoxins) (Barbetti *et al.*, 1996). Indirect losses include 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.*, 1996). Pasture legumes contribute to the saprophytic survival of fungal pathogens, including those of rotational crops, by providing the soil nitrogen necessary for soil survival. In particular, soils with low C:N ratios tend to favour saprophytic survival of several cereal and legume pathogens (Garrett, 1970).

### 1.3 Necrotrophic Fungal Root Pathogens

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Necrotrophic pathogens dominate regions with a winter-dominant rainfall, including those with a Mediterranean-type climate, because of the ease of survival of these trash-borne pathogens on infested residues through dry summer periods. The impoverished and nutrient-deficient soils across many parts of these regions are such that there is often little microbial competition with the necrotrophic pathogens, predisposing the plant host to these diseases (Sivasithamparam, 1993). Hence, it is not unexpected that necrotrophic fungal pathogens provide significant challenges to productive utilisation of annual medics, particularly in such regions. As global warming leads to climate change, there may well be significant changes in the relative importance of necrotrophic diseases, especially in some regions with Mediterranean-type climates such as southwest of Western Australia and significant areas of South Australia and Victoria (Chakraborty *et al.*, 1998). However, because of the ease of survival of these trash-borne pathogens on infested residues over hot and relatively rain-free periods, these potential effects from global warming are more likely to impact less upon necrotrophs than upon biotrophs that need a continuous green-bridge to maintain the pathogen cycle. Since the widespread adoption of minimum tillage practices in some regions, such as across southern Australia, burial and/or disposal of residues through tillage is now not common and has led to greatly increased quantities of infested host residues remaining, increasing disease pressure as has occurred for the trash-borne necrotrophic pathogen *Leptosphaeria maculans* (Barbetti *et al.*, 2000; Sivasithamparam *et al.*, 2005), that is responsible for blackleg disease on canola across southern Australia.

# Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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There have been some previous reviews addressing at least some soil-borne fungal and nematode pathogens in Australia (e.g. Barbetti *et al.*, 1986; Johnstone and Barbetti, 1987; Barbetti *et al.*, 1996). However, these previous reviews generally cover, at best, only some aspects diseases of annual or perennial pasture legumes and to our knowledge, soil-borne grass diseases have never been previously addressed. It is noteworthy that before 1970, diseases of pastures in Australia were considered not to be a significant problem and therefore did not warrant control measures (Sloane *et al.*, 1988).

## 2 Soil-borne Diseases of Clovers

### 2.1 Introduction

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Of the clovers utilised in Australia, subterranean clover (*Trifolium subterraneum*) is the most important pasture legume in temperate and Mediterranean regions of southern and eastern areas (Morley, 1961; Powell, 1970), and is has been established in over 20 million ha of dryland pasture (Cocks *et al.*, 1978; Gladstones, 1975). Subterranean clover is the most important pasture legume in temperature regions of southern and eastern Australia (Morley, 1961; Powell, 1970; Gladstones and Collins, 1983) particularly between latitudes 30 to 39°S and has been sown over an estimated area of 16 million ha or more of dryland pasture (Cocks *et al.*, 1978; Collins and Gladstones, 1984; Gladstones, 1975). Subterranean clover is the main basis of the improved pastures upon which southern Australia's animal industries depend and the nitrogen it fixes is the foundation for much of the country's cereal industry (Gladstones and Collins, 1983).

Particular advantages of subterranean clover as an annual pasture legume include substantial increases in stock carrying capacity, and increases in yields of subsequent cereal crops as a consequence of their ability to fix nitrogen. Other advantages include its ability to tolerate a variety of pasture managements, including heavy continuous grazing, and its outstanding effectiveness in preventing soil erosion. Additionally, the dense, rather shallow root system is highly effective for building up soil nitrogen, organic matter and physical structure. A final advantage of subterranean clover is its relative tolerance of waterlogging (Gladstones, 1975). Many other pasture legume and grass species are components of pastures in temperate Australia. Root diseases of subterranean clover has previously been extensively reviewed, but 20 years ago (Barbetti *et al.*, 1986).

### 2.2 Symptoms

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Root rots of subterranean clover are widespread in temperate and Mediterranean regions of 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. The disease can be evident as 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). In New South Wales, Stovold (1971) reported extensive rotting of the lateral feeder roots and stunted roots on affected plants. In Victoria, Clarke (1983c) described the root symptoms as tap roots with a dark brown to black wound about

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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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. 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). Irwin and Jones (1977) reported a root and stolon rot of white clover (*Trifolium repens*) in Queensland, involving brown discolouration of tap and lateral roots extending into the vascular tissue and often resulting in the total rotting of affected roots.

### 2.3 Impact

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Root rot has seriously adversely affected the production from subterranean clover pastures over the past 15-20 years (Johnstone and Barbetti, 1986).

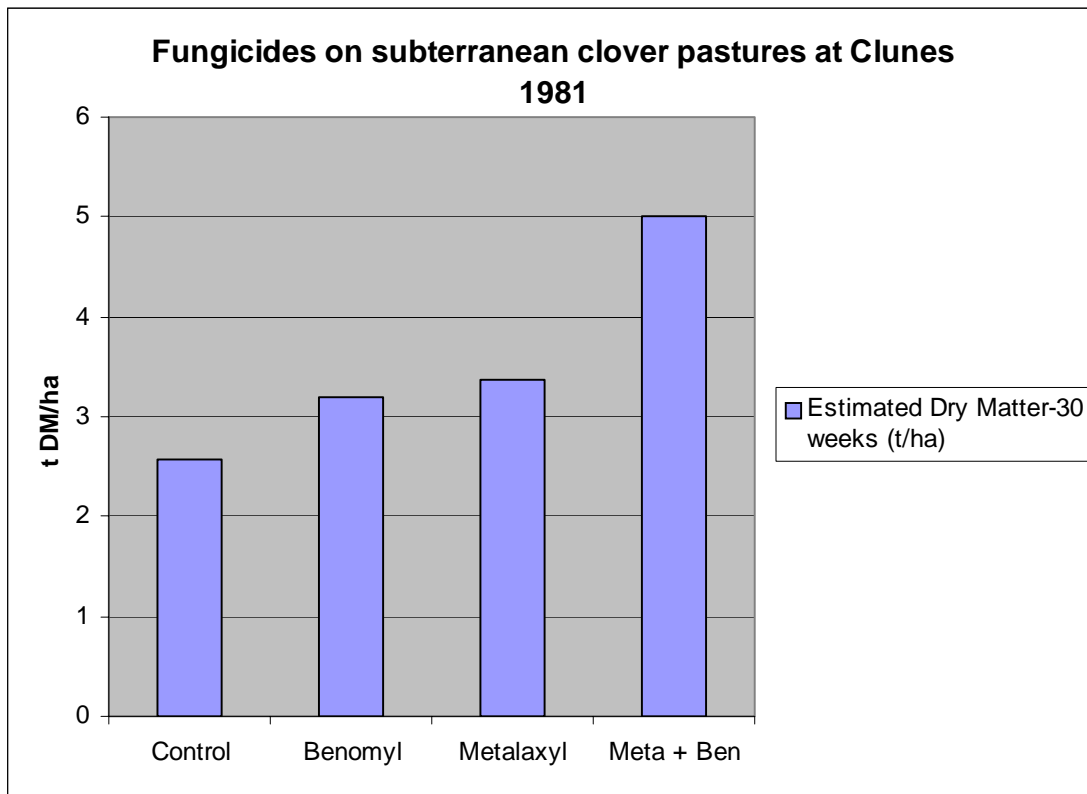
In Victoria, the problem was first recognized in 1960 (Anon., 1960) and is a serious problem which reduces the productivity of pastures (Taylor, 1984; Taylor *et al.*, 1984). A survey of subterranean clover pastures in 1970 (McGee and Kellock, 1974) showed that root rots were widespread in northern, 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.

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.

In Western Australia decline was first recognised by Shipton (1967). 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. He showed that rotting of the lateral root system had little effect on plant size, probably because most plants can rapidly produce new lateral roots to offset those damaged or lost from root rot.

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

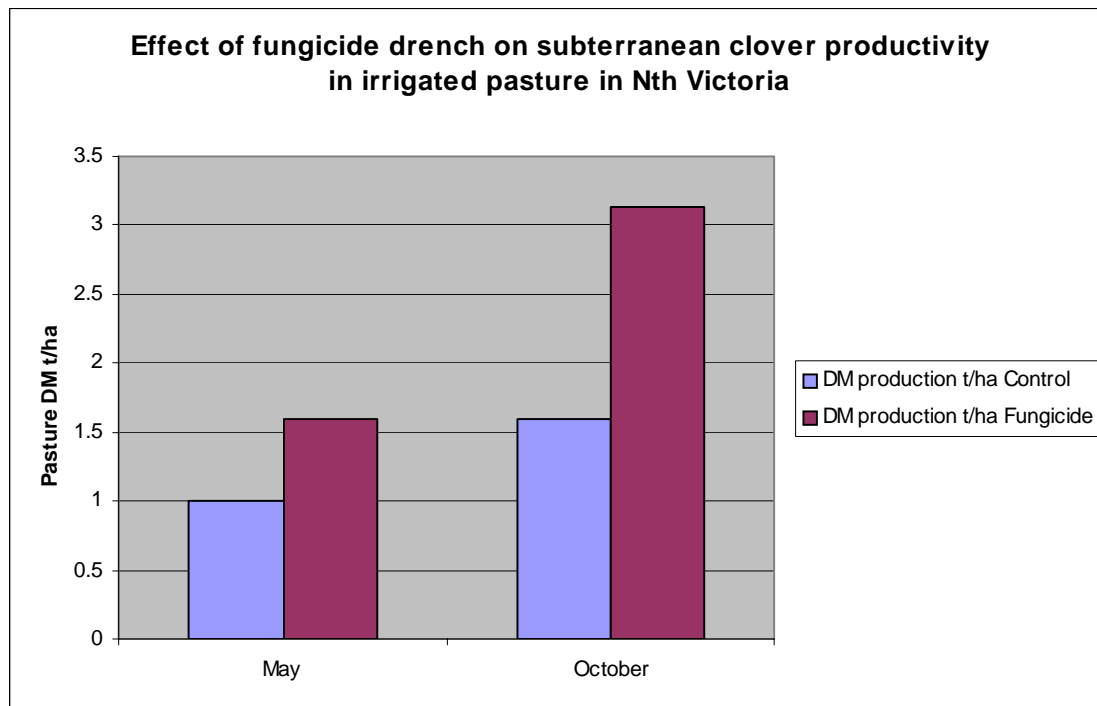
Greenhalgh and Clarke (1985) used drenches of fungicides metalaxyl (against *Pythium* and *Phytophthora*) and benomyl (against *Fusarium*) in 1981 to study significance and etiology of root rot of subterranean clover in dryland subterranean clover pastures in Victoria. They examined sites located at Clunes in Western Victoria north of Ballarat that consisted of acidic soils sandy loams of pH (in water) approximately 5.4 and they estimated the total herbage yields following fungicide drenches in order to determine the effects of fungicides on root pathogens and production in subterranean clover permanent pastures. Increases in subterranean clover herbage production following fungicide application are shown in Fig. 1.



**Figure 1.** Estimated total herbage yield increases in subterranean clover permanent pastures following application of drenches of fungicides metalaxyl (against *Pythium*) and benomyl (against *Fusarium*) in 1981 at Clunes in Western Victoria.

Taylor *et al.* (1983) investigated root rot of irrigated subterranean clover in northern Victoria, both its significance and the prospects of control. These studies were made utilizing irrigated subterranean clover pastures established in 1982 on a Wana silt clay-loam over heavy clay of pH (in water) 5.9 by application of fungicide drenches of metalaxyl and benomyl 2 days before the 1st irrigation and then measuring the dry matter production in May and again in October. Increases of nearly 60% in May and more than 95% in October in herbage production were obtained from where the fungicide application were made (see Fig. 2)

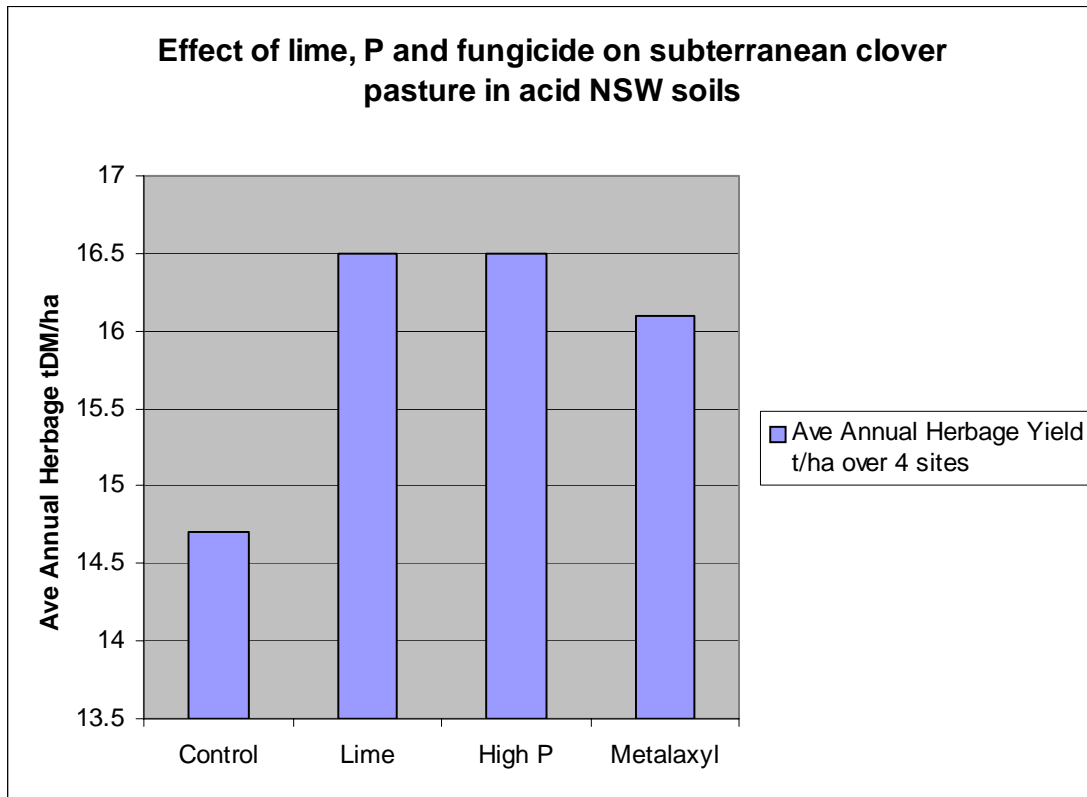
## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems



**Figure 2.** Dry matter production of subterranean clover irrigated pasture in May and again in October 1982 following application of fungicide drenches of metalaxyl and benomyl 2 days before 1st irrigation at the beginning of the season.

Hochman *et al.* (1990) examined the factors contributing to reduced productivity of subterranean clover pastures on acid soils at four field sites with soil acidity and poor clover growth (Holbrook, Yerong Ck, Oberne, Lankey's Ck) in NSW where soil pH (in water) ranged from 4.2 - 4.5. They utilized multiple treatments including P, Mo, lime pelleted seed, lime additions, metalaxyl (against *Pythium* and *Phytophthora*), ryegrass competition, lime+Mg+trace elements. Results showed increases in herbage yields of the order of 10-12% from these treatments (Fig. 3).

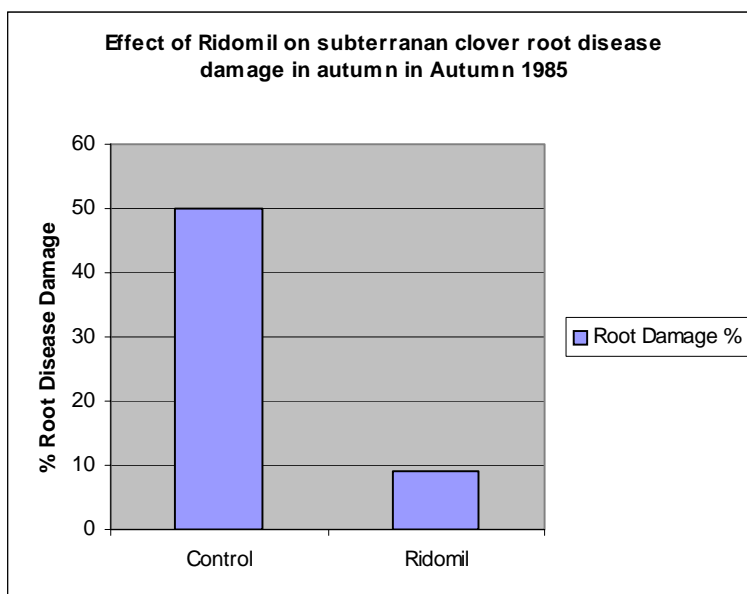
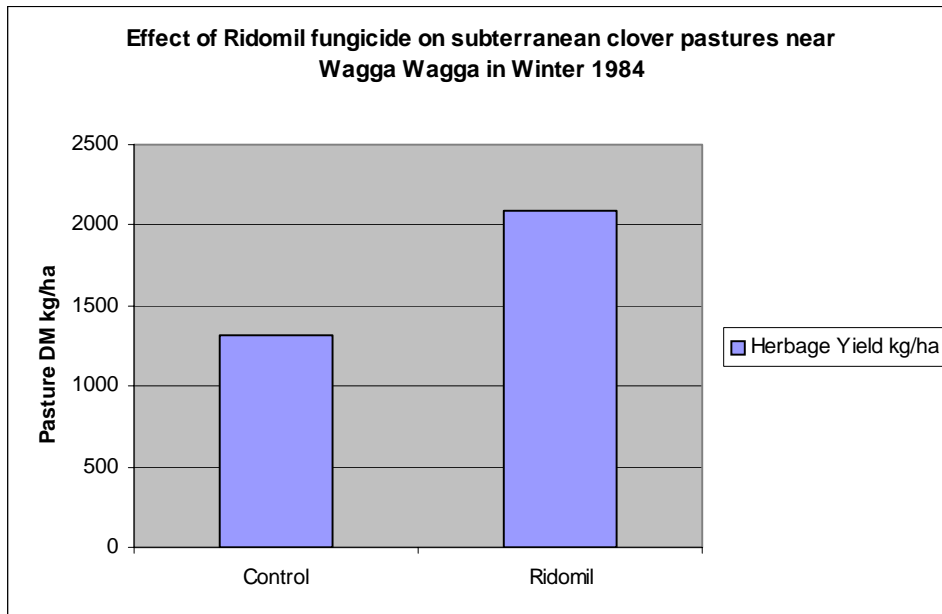
## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems



**Figure 3.** Increases in herbage yields of subterranean clover from application of multiple treatments including P, Mo, lime pelleted seed, lime additions, metalaxyl (against *Pythium* and *Pythophthora*), ryegrass competition, lime+Mg+trace elements.

In NSW, Dear *et al.* (1985), used applications of the fungicide Ridomil used to control *Pythium* and *Phytophthora* in subterranean clover pastures near Wagga and determined increases in herbage yield of up to 58% as a consequence of reductions of root disease damage of up to 82% from this fungicide application (Figs. 4a and 4b).

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems



**Figures 4a and b.** Increases in herbage yield (Fig. 4a) and decreases of root damage from disease (Fig. 4b) in subterranean clover pastures near Wagga Wagga following applications of the fungicide Ridomil used to control *Pythium* and *Phytophthora*.

### 2.4. Fungal Pathogens Involved

A number of soil-borne fungi have been shown to be associated with root rot. In the first record of root disease in Victoria (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.*



## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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*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. roseum* "Culmorum" and *F. roseum* "Gibbosum", 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 were involved in root disease of subterranean 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), 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%, particularly in long growing seasons and in irrigated areas in Victoria. In Victoria, (Taylor *et al.*, 1985b; Greenhalgh and Flett, 1987; Taylor and Greenhalgh, 1987). *P. clandestina* was shown to be an important root pathogen of subterranean clover in NSW by Dear *et al.* (1993).

In Western Australia, the presence of *P. clandestina* was confirmed in 1984 (Taylor *et al.*, 1985a). Barbetti (1989d) reported that the most susceptible cultivars 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.

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.

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).

In Western Australia, Shipton (1967) 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

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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pathogenic while *F. oxysporum* and *Phoma medicaginis*, particularly when tested singly, were only weakly pathogenic. 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.

In Western Australia, *F. avenaceum*, *Leptosphaerulina trifolii*, *Myrothecium verrucaria*, and *P. medicaginis*, commonly isolated from subterranean clover foliage, are all reported to cause some root rot under non-competitive conditions (Barbetti, 1984c). Broughton (1983) showed that while *Phytophthora cinnamomi* can colonize and infect subterranean clover growing in infected soils, plants rarely showed disease symptoms. In Western Australia, 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. 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.

Studies with *P. clandestina* in the 1980's assumed the existence of only a single race of *P. clandestina*. However, a later study by Flett (1994) of ten *P. clandestina* isolates on 5 subterranean clover cultivars, showed that cultivars 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 cultivars and up to 112 isolates of *P. clandestina* collected from different geographic regions across southern Australia. However, recently, You *et al.* (2005a) screened 101 isolates of *P. clandestina* from Western Australia on 9 subterranean clover cultivars 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 Australia, 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 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 to *P. clandestina* race delineation was clearly conservative and different from previous studies and will facilitate, for the first time, rapid recognition and characterization of the races, including their pathogenicity in relation to the differentials.

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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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 cultivar resistances.

In comparison to Western Australia, Victoria and N.S.W., there have been very few reports of investigations undertaken in South Australia or Queensland 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. In Queensland Irwin and Jones (1977) consistently isolated *P. middletonii* from necrotic stolons and roots of *Trifolium* spp.

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 eutichies*, *Rhizoctonia* species and *F. avenaceum*. However, it is likely that additional fungi will be found associated with diseased roots in the future as new isolation procedures are applied. Enhanced pathogenesis due to phytotoxins in soil has been reported for a variety of pathogens (Harris and Kimber, 1983) and this may also have a role in the predisposition of subterranean clover to root rots. This area therefore needs further investigation. The same may be true for *Cylindrocarpon didymum* which is found in south-western Western Australia (Barbetti, 2005), a pathogen that produces brefeldin A *in vitro* and the frequently observed stunted appearance of affected tap and lateral roots could possibly be a consequence of production of the highly toxic mycotoxin brefeldin A in the root tissue by this pathogen.

### 2.4 Environmental Influences

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Very few attempts have been made to investigate the relevance or importance of environmental factors in relation to the severity of root rots. In Western Australia analysis of climatic data for centres along the south coast from 1972 to 1975 showed that 1973, a particularly severe root rot year, had significantly heavier and more frequent rain after the break of season than did the other years when root rot severity was much less (MacNish *et al.*, 1976). Also in Western Australia, Wong *et al.* (1984) investigated the effects of soil temperature (10, 15, 20, and 25°C) and moisture (45% water holding capacity (WHC), 65% WHC, and flooding) on the pathogenicity of *F. avenaceum*, *F. oxysporum*, *P. medicaginis*, *P. irregulare*, and *R. solani*, both alone and in combinations. The fungi investigated caused root rot over the range of soil temperatures and moisture conditions, conditions approximating those occurring in root rot-affected fields. Both soil temperature and soil moisture had marked effects on the disease severity and their effects varied with individual fungi and their combinations. The most severe root rotting occurred at 65% WHC, with less at 45% WHC, and

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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least under flooding conditions. There was often a significant interaction between temperature and moisture for the various fungi and fungal combinations tested. Subsequently, Wong *et al.* (1986c) demonstrated that *P. clandestina* could cause pre- and post-emergence damping-off in subterranean clover under a range of soil temperature (10, 15, 20 and 30°C) and moisture (65 and 100% WHC and flooding) conditions in a glasshouse. They showed that the greatest reductions in seedling survival occurred in saturated and flooded soil conditions. Most severe root disease occurred at a soil temperature of 10, followed by 15 and 20°C. *P. clandestina* interacted with *F. oxysporum*, but not with *F. avenaceum*, *P. medicaginis*, *P. irregulare* or *R. solani*, to produce more severe root rot than did each fungus alone. In the same way, for studies of foliage fungi as root pathogens, Barbetti (1984b) demonstrated the importance of temperature on the severity of root rot developed in pathogenicity tests, and that the temperature thresholds vary with different pathogens and/or their combinations.

Maughan and Barbetti (1983) showed that a fluctuating soil moisture of 45 to 65% WHC resulted in more severe Rhizoctonia root rot in white clover compared to a constant soil moisture of 65% WHC. This indicates that the stressing of plants exacerbates the pathogenicity.

In a study by Barbetti (1990a), the addition of lime affected seedling survival and the levels of rotting of tap and lateral root systems by *F. avenaceum*, *P. clandestina*, *P. irregulare* and *R. solani*, four known root pathogens of subterranean clover in Western Australia. The different root pathogens often responded differently to the addition of lime. The relative cultivar resistance-rankings to individual pathogens sometimes varied widely depending on the amount of lime added. There was clearly an interaction between lime and cultivar and between lime and pathogen for all parameters measured and the potential for utilising addition of lime for disease control warrants further investigation

Barbetti (1984d), using intact cores taken from Western Australian root rot-affected fields, detected slight reductions in root rot severity but no effect on the incidence of various root rot fungi, from prior application of a paraquat/diquat mixture in the form of commercial Spray-seed<sup>®</sup>.

Wong *et al.* (1986b) investigated the effects of temperature, pH and water potential on the growth or survival of *P. clandestina*. On agar it grew over a pH range of 4-9 and growth rate increased as the pH of the medium rose from 4 to 6 at temperatures of 15, 20, and 25°C. There was a marked reduction in growth with increasing water stress at all temperatures. Generally, growth rate increased with progressive increase of incubation temperature up to 20°C. More recently, Barbetti (2005) demonstrated that *Cylindrocarpon didymum* caused most disease at the cooler temperature regime of 15/10°C, a temperature regime that would closely approximate field soil temperatures in the lower south-west of Western Australia during the winter months where this pathogen appears to be most common. Whether this pathogen occurs in other areas of southern Australia needs to be determined.

An osmotic water potential of -12 bars prevented mycelial growth and best growth was at 0 bar for all temperatures for *P. clandestina*. Saprophytic survival of the pathogen in pasteurised soil was best under cooler conditions (5-10°C) at all water potentials tested (-0.01, -0.026, and -0.063 bars). At higher temperatures (15-32°C) *P. clandestina* survived better under wetter soil conditions. Studies on the detection of *P. clandestina* on infected subterranean clover roots with time (Wong, 1986) showed that the fungus was detected most readily two weeks after germination, the activity gradually declining after this phase. Wong *et al.* (1986a) studied the nature and behaviour of *P.*

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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*clandestina* in soil and showed that the pathogen could only be recovered from soil screenings 250-499  $\mu\text{m}$  and 500  $\mu\text{m}$ -0.99 mm containing small root fragments. In soil the introduced inoculum of the fungus was incapable of saprophytically and competitively colonizing dead subterranean clover cotyledons. Exposure of *P. clandestina* inoculum to increasing numbers of microbes, by adding greater proportions of non-sterile field soil, reduced the severity of root rot of subterranean clover. *P. clandestina* was able to spread between 15-30 mm through pasteurized soil within a period of 20 days. It was demonstrated that the most probable number for *P. clandestina* propagules in a root rot-affected field in Western Australia was greater for the period January to June compared to July to December, and that it increased after January to peak in May, the month in which root rot is often severest in this region (Wong *et al.*, 1986b). These studies also showed that the root disease index was, however, negatively correlated ( $P < 0.05$ ) with disease suppressiveness index linearly ( $r = -0.76$ ) and quadratically ( $r = -0.81$ ). Subterranean clover appears to be the sole source of *P. clandestina* inoculum in the pasture sward of mixed plant species not containing other pasture legume species.

You *et al.* (2006) demonstrated that rainfall was an important determinant of race distribution for *P. clandestina*, with the majority and most diverse of *P. clandestina* races occurring in the 700-1000 mm rainfall zone. Race 173 occurred across all rainfall zones = 300 mm and race 177 in all zones = 400 mm. These differences are important in relation to the selection/breeding of cultivars for specific regions of Western Australia where particular races occur.

Environmental factors such as rainfall, soil moisture and soil temperature have been clearly shown to have a marked effect on both the disease severity from individual pathogens and on the interactions that occur between the different root pathogens. Further investigations in this area may help explain some of the differences in symptoms, disease severity etc. that occur between different regions and this may warrant further investigation. In eastern Australia, Smiley *et al.* (1986), using intact field cores, showed that root rot was mild on plants in continually moist cores at 10°C, and severe in cyclically wetted and dried cores at 10, 15 and 20°C, and in continually moist cores at 15 and 20°C. They also demonstrated that removal of seedling leaves, to simulate grazing, accentuated root rot severity.

### 2.5 Seed-borne Fungal Pathogens

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Certain fungi associated with root rot can be seed-borne. McGee and Kellock (1974) showed that both *F. avenaceum* and *F. oxysporum* were commonly associated with seed in Victoria, with *F. avenaceum* being strongly pathogenic and *F. oxysporum* only weakly pathogenic. Kellock *et al.* (1978) surveyed fungi associated with *Trifolium* and *Medicago* seed lines and isolated *F. arthrosporioides*, *F. avenaceum*, *F. equiseti*, *F. acuminatum*, *F. culmorum*, and *F. oxysporum* from seed. In laboratory tests only isolates of *F. avenaceum* were pathogenic on subterranean clover roots. Later Kellock *et al.* (1980) showed that *F. avenaceum* was carried in the hilum of the subterranean clover seed. MacNish (1977) surveyed fungi associated with subterranean clover seed in Western Australia and found that *F. roseum* "Avenaceum" was associated with some seeds. *F. roseum* (other than "Avenaceum") and *Rhizoctonia* spp. were present in low numbers, and *F. oxysporum* was the fungus most frequently isolated. He concluded that the low levels of *F. roseum* "Avenaceum" and the absence of *Pythium* spp. on seeds, coupled with the fact that there was little or no relationship between root rot incidence and fungi associated with seed, was an indication that seed infection is unimportant in the aetiology of root rots of subterranean clover in Western

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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Australia. This view is supported by the investigations of Barbetti (1984a) who showed that removal of fungi from subterranean clover seed had no influence on root rot levels in subsequent field plantings.

### 2.6 Soil-borne Nematodes

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The role of nematodes in producing root disease, both in their own right and in complexes with fungal pathogens, needs further investigation. The earliest reports of nematode damage to subterranean clover are of root knot nematode in Australia (Ludbrook *et al.*, 1953). In Australia, a wide range of nematode genera have been recorded in association with subterranean clover roots, including *Aglencus*, *Aphelenchus*, *Ditylenchus*, *Filenchus*, *Meloidogyne*, *Merlinius*, *Neopsilenchus*, *Nothotylenchus*, *Pratylenchus*, *Rotylenchus* and *Tylenchorhynchus* (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, 1967). More recently, 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 The South-East, SA provides a contrast. 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.

However, *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 New South Wales (Colman, 1964). Powell (1971) demonstrated that significant interactions occur between nematodes and fungi in root disease complexes. Elsewhere, Nordmeyer and Sikora (1980) demonstrated a nematode by *F. avenaceum* interaction on subterranean clover roots. *Meloidogyne* spp. can be considered the most potentially problematic nematode parasites of subterranean clover as highlighted by the study of Kouame *et al.* (1989), which showed that 134 cultivars were susceptible to and damaged by *M. arenaria*, *M. incognita* and *M. javanica*.

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

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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five inoculum levels (0, 800, 1 600, 4 000 and 8 000 eggs per 250 ml pot) did not demonstrate clear pathogenicity, they showed that it is pathogenic at higher inoculum levels (> 16 000 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. Reports and data from other Australia States are more limited.

In addition to the above, it is worth noting that *Heterodera daverti* is considered a damaging pathogen of subterranean clover in Tunisia and 13 susceptible cultivars were identified (Nordmeyer *et al.*, 1978). Given *H. daverti* is not known to occur in Australia, this highlights the possibility that further exotic pathogens of subterranean clover could be introduced. Similarly, subterranean clover is a host for *H. trifolii* (Marcela-Yanez *et al.*, 1999), which occurs in Australia, so it is possible that some species might emerge in the future as more significant constraints to subterranean clover than at present.

### 2.7 Soil-borne Pathogen Interactions with Viruses

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Interactions with virus disorders also need to be considered. Such interactions are known to occur between viruses and *Fusarium* species on red clover (*Trifolium pratense*) (Denis and Elliot, 1967). A survey conducted by Mekwatanakarn (1985) indicated that a high incidence of both virus

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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(particularly alfalfa mosaic virus and white clover mosaic virus) and root rot in white clover in Western Australia. A wide range of viruses occur on subterranean clover and damage has particularly been caused by subterranean clover stunt virus and soybean dwarf virus (Johnstone and Barbetti, 1986). It is likely that significant interactions between viruses and root-rot fungi occur for subterranean clover pastures but these have yet to be defined.

### 2.8 Disease Management Using Chemicals

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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 that caused by *P. clandestina*, as did Greenhalgh *et al.* (1994) for applications of potassium phosphonate, primarily against this same pathogen.

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 cultivars 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, 1985) 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 (M.J. Barbetti, unpubl.). In contrast, various metalaxyl seed and soil drench treatments had no significant beneficial effect on the number of plants germinated, root rot indices, or total plant dry weights (Barbetti, 1983a,c, 1984b, 1985b).



## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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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. However, it is clear from the above studies that development of effective seed treatments is possible and could certainly help in the successful reseeded and re-establishment of subterranean clover into root rot deteriorated pastures. This possibility needs further investigation and testing of newer generation fungicides is warranted.

### 2.9 Disease Management Using Cultural Practices

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Field experiments in the 1970s in south western 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 reseeded, or spring cultivation before sowing to oats followed by a March cultivation and reseeded. 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. 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.

Small reductions in root rot severity have been obtained from inoculating seed with rhizobia (M.J. Barbetti, unpubl.). A strain of *Rhizobium trifolii* significantly reduced root rot caused by *F. avenaceum* in glasshouse studies (Wong, 1986). In the same way, Smiley *et al.* (1986) also demonstrated that root rots could be reduced by treatment of seeds with rhizobia on legumes.

The effects of soil nutrition and pH on the severity of root disease are probably also important. The productivity of pastures in southern Australia has improved tremendously over the past fifty years through the application of fertilizers, particularly superphosphate. However, this has led to

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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increasing soil acidity problems in many areas making conditions less favourable for plant growth (Donald and Williams, 1954; Lee, 1980; Williams, 1980) and more conducive to damage from plant pathogens particularly those causing root rot (Stovold, 1983).

In two field trials in Western Australia (Barbetti, 1991), 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. This prospect warrants further investigation.

### 2.10 Disease Management Using Host Resistance

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Extensive field testing of subterranean clover cultivars 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. Cultivars with good field resistance to root rot are Daliak (Gillespie, 1983a), Dinninup (Gillespie, 1983a), Esperance (Nicholas, 1980b), Junee (Nicholas, 1985a), and Karridale (Nicholas, 1985b). Larisa is known to have a moderate degree of field root rot resistance (Nicholas, 1980a) while Trikkala has less but still useful resistance (Nicholas, 1980c). Cultivars Denmark (Nichols and Barbetti, 2005b) York (Nichols and Barbetti, 2005h) and Goulburn (Nichols and Barbetti, 2005d), all released in the 1990's, all have resistance to the most commonly occurring race of *P. clandestina*. Cultivar Gosse (Nichols and Barbetti, 2005c) released in the 1990's, Riverina (Nichols and Barbetti, 2005f), along with the most recently released cultivars of Napier (Nichols and Barbetti, 2005e) and Coolamon (Nichols and Barbetti, 2005a), all have good resistance to more than one of the most common races of *P. clandestina*. Unfortunately, the recently released cultivar Urana is quite susceptible to the two most commonly occurring races of *P. clandestina* (Nichols and Barbetti, 2005g). In attempts to refine methods of screening subterranean clover cultivars for resistance to root rot Barbetti *et al.* (1986) screened 12 commonly grown cultivars 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 cultivars 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 cultivars generally differed in their response to the five pathogens and for any one pathogen there was generally a

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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range of cultivar susceptibilities. Cultivars 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.

More recently, 84 genotypes, including 71 ssp. *subterraneum* and ssp. *yanninicum* breeding lines of subterranean clover and 13 commonly used cultivars were screened by You *et al.* (2005b) in the glasshouse for resistance to root rot caused by two races of *Phytophthora clandestina* that occur most widely in Australia. Resistance to race 001 was identified in seven mid-season genotypes of ssp. *subterraneum*, including the new cultivar, 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 cultivar, Napier, 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 cultivars to minimise damage from this disease or as parents in breeding programs to develop cultivars with improved resistance to *P. clandestina*. This study established the availability of 51 advanced lines and 11 cultivars as sources of resistance against *P. clandestina* race 001 and 36 lines and 4 cultivars for race 373, among these 36 lines and 4 cultivars 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.

The recent work of You *et al.* (2005d) to characterise a total of eleven races (in contrast to the 5 recognized previously) presented the first clear picture of the racial distribution of *Phytophthora clandestina* in Western Australia and this will be important both to breeders and to farmers. Differences that were found in the distribution of race populations between Australian states will provide a sound basis not only for the selection/breeding of appropriate cultivars for specific regions of Australia to counter the predominant race populations, but also as a basis for enforcing quarantine measures in relation to seed movements within and outside Australia. This work is one of the few research investigations ever undertaken which indicates a clear evolution in pathogenic specialization of a pasture pathogen which could be related to the use of specific cultivars or spectrum of cultivars of a specific pastures species in a given geographic region. However, if we are to manage the significant threat posed to subterranean clover by *P. clandestina*, there is an urgent need to reassess across southern Australia the race situation for this pathogen which has the ability to rapidly generate new races to overcome existing cultivar resistances.

You *et al.* (2005c), also recently screened subterranean clover breeding lines for resistance to root rot caused either by *F. avenaceum* or *P. irregulare*. One of the tested lines showed good resistance to root rot caused by *P. irregulare*, viz. the late maturing *T. subterraneum* ssp. *subterraneum* line SL016. High levels of resistance to *F. avenaceum* were identified in 8 among 20 tested *T. subterraneum* ssp. *subterraneum* midseason lines, viz. SM005, SM006, SM007, SM009, SM014, SM015, SM016 and SM017; 5 among 17 tested late season lines, viz. SL004, SL006, SL009, SL010 and SL014; and 6 among 13 late season *T. subterraneum* ssp. *yanninicum* lines, viz. YL001, YL002, YL004 YL008, YL010 and YL011. These sources of resistance will be of significant value to breeding programs aimed at developing new more resistant cultivars to this particular pathogen as few sources of useful resistance in subterranean clover to this pathogen have previously been

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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identified. However, seedling survival in 14 among 17 late season and 15 among 20 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 midseason *T. subterraneum* ssp. *subterraneum* lines that showed no significant reduction in seedling survival in the presence of either *P. irregulare* or *F. avenaceum*. For five late season lines of *T. subterraneum* ssp. *subterraneum* viz. SL012, SL013, SL014, SL015 and SL016 and 6 midseason lines of *T. subterraneum* ssp. *subterraneum*, viz. SM003, SM009, SM010, SM012, SM018 and SM019 seedling survival was not significantly reduced in the presence of *P. irregulare*. It is noteworthy that specific resistance to root rot 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 rot but also on overall ability or tolerance of seedlings to survive in the presence of these pathogens. Resistance identified to either pathogen could be utilized in developing new cultivars for areas prone to the root rot caused by these pathogens or utilized as parental materials in breeding programs.

The most promising avenue for disease control so far has clearly been the development and use of cultivars with increased field resistance to root rot. Identifying sources of resistance to individual root pathogens should allow the development of cultivars with further enhanced root rot resistance. Ideally, identification of resistance to more than one pathogen in a single line or cultivar is ideal. This was recently demonstrated by You *et al.* (2005a) when screening 100 subterranean clover genotypes including 72 advanced breeding lines from *Trifolium subterraneum* ssp. *subterraneum* and *Trifolium subterraneum* ssp. *yanninicum* and 28 *Trifolium subterraneum* commercial cultivars 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 *Phytophthora clandestina*, *Pythium irregulare*, and *Fusarium 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 cultivars 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 with subterranean clover and also for other pasture species.

While fungicide treatments and manipulation of management practices are not effective enough to make their use economically justifiable for very susceptible cultivars, it is possible that they may have a place in an integrated control system incorporating cultivars with at least some resistance to root rot.

### **3 Soil-borne Diseases of Annual Medics**

Annual *Medicago* spp.(annual medics) are an important component of pastures in dryland farming world-wide (Walsh *et al.*, 2001), especially in the regions characterised and dominated by winter rains (Piano and Francis, 1992; Sheaffer and Lake, 1997), such as those with a Mediterranean type climate. Dryland farming systems in Australia are suited for annual medic-based pastures, which have been successful not only as a forage legume but also as a rotation option with grain crops.

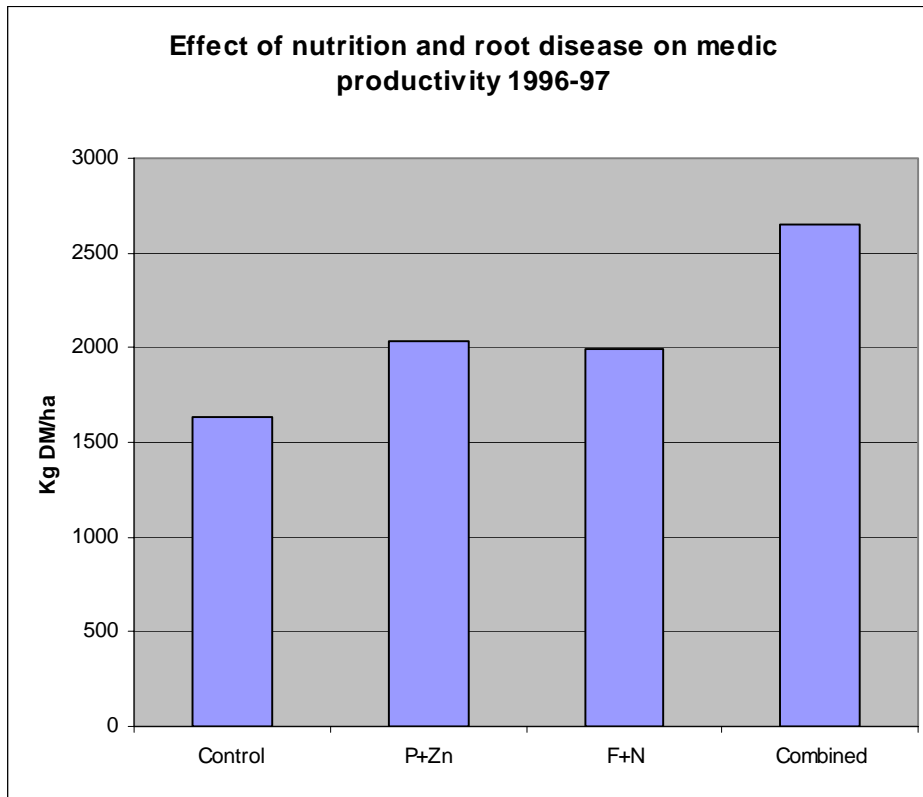
In southern Australia, annual medics have shown distinct advantages, such as high proportion of hard seed, that make them attractive over other annual legume species such as subterranean clover in many areas. Across southern Australia, annual medics are widely utilised (Chatterton and Chatterton, 1996) and there they are an important component of agricultural systems involving self regenerating pastures, in their own right and in association with ley farming and cropping regimes (Puckridge and French, 1983), and often provide an excellent 'disease break' for rotational crops that assists in reducing disease problems (Walsh *et al.*, 2001), providing grass weeds are controlled. The appropriate adaptation of some annual medics has led to rapid expansion in the areas sown in Australia, for example, in Western Australia alone, some 1 million ha of annual medics pastures (particularly *M. polymorpha* var. *brevispina* cultivars) form an important component of dry-land farming systems with the potential expansion of a further 3 to 4 million ha suitable for growing annual medics pastures (M. A. Ewing, personal communication). This has primarily been due to the development of acid tolerant *Rhizobium* (Robson and Loneragan, 1970; Howieson and Ewing, 1986, 1989) and such development opens up enormous potential for further expansion of annual medics into regions where acid soils predominate (Vincent, 1988; Piano *et al.*, 1991; Howieson *et al.*, 1988; Piano and Francis 1992).

#### **3.1 Impact of Medic Root Diseases**

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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 (Fig 5).

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems



**Figure 5.** Showing the relative importance of soil nutrition and root disease in the medic decline syndrome. Taken from Belotti, W, 'Medic Decline Syndrome - Problem Definition', (GRDC Final report, June 1998).

### 3.2 Necrotrophic Fungal Foliar and Root Pathogens

#### 3.2.1 Pathogen survival between seasons

Necrotrophic soil- and trash-borne pathogens dominate regions with a winter-dominant rainfall, including those with a Mediterranean-type climate, because of the ease of survival of these pathogens in infested soil and plant residues through dry summer periods (Sivasithamparam, 1993). Hence, it is not unexpected that necrotrophic soil-borne fungal pathogens provide significant challenges to productive utilisation of annual medics, particularly in such regions. However, because of the ease of survival of these pathogens, both as trash-borne on infested residues and as soil-borne pathogens, over hot and relatively rain-free periods, the potential effects from global warming are more likely to impact less upon necrotrophs than upon biotrophs (e.g., mildews and rusts) that need a continuous green-bridge to maintain the pathogen cycle. Since the widespread adoption of minimum tillage practices in some regions, such as across southern Australia, burial and/or disposal of residues through tillage is now not common and has led to greatly increased quantities of infested host residues remaining.

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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### 3.2.2 Impact of necrotrophic pathogens causing root and/or foliar (e.g., crown and stem) diseases

There are necrotrophic pathogens of annual medics that have both a clear soil-borne and foliar-borne roles in reducing medic productivity (e.g., *Phoma medicaginis*, *Colletotrichum*), are examples of two of the most important and/or frequently occurring diseases caused by such particular necrotrophic fungi are as follows:

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, 1983, 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 is posing a potential threat to susceptible cultivars (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, 1983). Phoma black stem disease is also one of the most frequently occurring and important diseases on annual medics in Europe, North America and South Africa (Sampson and Western, 1941; Graham *et al.*, 1979; Lamprecht and Knox-Davies, 1984b; O'Neill *et al.*, 2003).

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), either *C. trifolii* and/or *C. destructivum* in South Africa (Lamprecht, 1984b,c, 1986b; Lamprecht and Knox-Davies, 1984b), and by *C. trifolii* in both North America (O'Neill and Bauchan, 2000) and Europe (Raynal, 1977; Troeung and Gosset, 1990).

### 3.2.3 Importance of necrotrophic pathogens from their side effects

Phyto-oestrogen production can be an important side effect of some necrotrophic pathogens causing root and/or foliar (e.g., crown and stem) diseases. For example, some diseases caused by necrotrophic fungi commonly occurring in Australia (e.g., Phoma blackstem) on annual medics are also known to stimulate production of phyto-oestrogenic compounds, such as coumestrol (Francis and Millington, 1971; Collins and Cox, 1984; Barbetti and Fang, 1991; Barbetti and Nichols, 1991b) to levels between 300 and 550 ppm in stems and/or burrs in ungrazed swards, and levels in excess of 1000 ppm have been recorded for *M. tornata* cv. Swani (Barbetti and Fang, 1991; Barbetti and Nichols, 1991b; Barbetti, 1993a, 1995a). It is likely that total phyto-oestrogen production could have been much higher than the recorded coumestrol contents in the above studies, as levels of other coumestans likely to be present, such as 4'-O-methyl coumestrol (Bickoff *et al.*, 1965), were not measured in those studies. It is probable that the production of phyto-oestrogens provides some selective advantage to the host by providing protection against attack by insects, especially in harsh regions with a winter-dominant rainfall, including Mediterranean-type climate regions, as opposed to less harsh environments.

Significant amounts of these phyto-oestrogenic compounds adversely affect ovulation rates in sheep (Smith *et al.*, 1979; Croker *et al.*, 1994a, 1994b, 1999, 2005). Even coumestrol levels as low as 25 ppm in the diet have been shown to adversely affect the ovulation rate in sheep (Smith *et al.*, 1979). Response to disease varies between accessions of annual medics which are worse in the

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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production of phyto-oestrogens (Barbetti and Fang, 1991; Barbetti and Nichols, 1991b). A survey of annual medic pastures in Western Australia indicated that very high levels of both foliar disease and coumestrol can sometimes occur (Croker *et al.*, 1994a,b). However, there is also a suggestion that other non-disease factors, such as soil type (Francis and Millington, 1971) and especially P nutrition (Marshall and Parkin, 1970), can also be involved in stimulating production of phyto-oestrogens in annual medics

### 3.2.4 Seed and burr health

*Fusarium* species appear to be quite common in Australia in annual medic seed (Kollmorgen 1974; Wan Zainun and Parbery, 1977; Kellock *et al.*, 1978; Mebalds, 1983) and burrs (Barbetti and Allen, 2005, 2006). *F. acuminatum*, *F. avenaceum*, *F. equiseti*, *F. chlamydosporum* and *F. graminearum* have all been reported to be toxigenic fungi in Australia (Barbetti and Allen 2006). Some Australian isolates of *F. graminearum* are confirmed producers of nivalenol and/or deoxynivalenol (Blaney and Dodman, 1988; Tan *et al.*, 2004). The studies by Barbetti and Allen (2005, 2006), confirm a strong association of one or more of *F. avenaceum*, *F. equiseti*, *F. chlamydosporum*, and *F. graminearum* being associated with sheep feed refusal and/or reduced feed intake situations Western Australia. This highlights the fact that the impact of necrotrophic pathogens on annual medics in Australia is not confined to simple effects on plant yield limiting factors.

Countries outside of Australia also have reported similar *Fusarium* spp. associations with seed and/or burrs, such as South Africa (Lamprecht *et al.*, 1986c, 1988, 1990). *F. acuminatum*, *F. avenaceum*, *F. equiseti*, *F. chlamydosporum* and *F. graminearum* have also been reported to be toxigenic in South Africa (Lamprecht *et al.*, 1986c). *F. acuminatum*, *F. avenaceum* and *F. graminearum*, are known to produce the mycotoxin moniliformin (Rabie *et al.*, 1982; Lamprecht *et al.*, 1986c), *F. acuminatum* and *F. graminearum* also produce diacetoxyscirpenol, while *F. equiseti* produces zearalenone (Lamprecht *et al.*, 1986c), all mycotoxins with severe adverse effects on animals if ingested in sufficient quantities.

### 3.3 Soil-borne Diseases – Affect Critical Functions of Annual Medic Roots

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There are also a number of necrotrophic soil-borne pathogens that have been associated with productivity decline in annual medic pastures. For example, decline of annual medic pastures has been reported throughout the cropping zones of southern Australia (Neal *et al.*, 1997) where their value in crop rotations has been significantly reduced (Bretag, 1985). Several studies have been conducted to determine decline of annual medic pastures in Australia (Andrew, 1962; Kollmorgen, 1974; Kellock *et al.*, 1978; Bretag and Kollmorgen, 1986; Mebalds, 1987; Barbetti, 1989c; You *et al.*, 1999, 2000). Kollmorgen (1974), Bretag (1985) and Mebalds (1987) implicated root disease to be critical in this decline. A variety of root diseases have been associated with this decline across southern Australia. Andrew (1962) implicated damping-off of annual medic seedlings during the early post-emergence period caused by *Pythium* spp., especially in *M. minima*, while Kollmorgen (1974) showed that it was mainly *F. avenaceum* that reduced both emergence and dry weight of plants of *M. truncatula* that survived the seedling emergence period.

Decline of annual medic pastures has also been reported for the winter rainfall region of South Africa (Lamprecht *et al.*, 1988), where a number of soil-borne pathogens, including species of *Fusarium* such as *F. avenaceum*, *F. culmorum*, *F. graminearum* and *F. lateritium*, various *Pythium* spp. such as *P. irregulare*, *P. ultimum* and *P. spinosum* (Lamprecht *et al.*, 1988) and *Cylindrocladium*



## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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*scoparium* (Lamprecht, 1986a) have been implicated. The similarities in necrotrophic fungal pathogens involved in such decline in Australia and South Africa is not surprising as both share similarities, such as in having extensive areas of impoverished soils with low microbial buffering capacity. Such diseases have also been reported from the United States of America (Thies, 1991; Zhu *et al.*, 1996; Haan *et al.*, 2002).

*Phytophthora clandestina*, recorded to date only in Australia, is known to attack some annual medics such as *M. truncatula* (Clarke and Greenhalgh, 1986; Barbetti, 1989c), and also, but to a lesser extent, *M. rugosa* and *M. scutellata* (Clarke and Greenhalgh, 1986). Following the initial work of Taylor *et al.* (1985) to identify the first race of *P. clandestina* in Western Australia, more recent studies by You *et al.* (2005b), identified that there were 10 races of *P. clandestina* present there. 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. In the United States of America, *Phytophthora* root rot, caused by *Phytophthora medicaginis*, is common throughout the upper mid-west (Thies, 1991) and host resistance is necessary in germplasm utilised in that region (Haan *et al.*, 1996, 2000, 2002). This pathogen may prefer wetter conditions than those available in much of southern Australia and perhaps this is why, to date, this particular pathogen has not been reported as a problem in this particular region. If surveys conducted across southern Australia can confirm area freedom from this pathogen and also for the foliar pathogen *Leptotrochila medicaginis*, then quarantine measures may be useful to prevent their introduction into annual medic and perennial lucerne pastures of southern Australia.

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). Root rots caused by several 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 south-western Western Australia, surveys of annual medic pastures on 116 farms in the grain belt during 1996 and 1997 indicated that soil-borne pathogens such as species of *Pythium*, *Fusarium* and *Phoma* pose a threat to annual medic pastures (You *et al.*, 2000). However, the extent of this threat was not quantified in these studies.

As with their association with annual medic burrs, *Fusarium* species on roots and crowns are cause for additional concern as a number of *Fusarium* species have been shown to be responsible for the production of mycotoxins deleterious to livestock, both in Australia (Barbetti and Allen, 2005, 2006) and also in South Africa (Lamprecht *et al.*, 1986c).

### 3.4 Plant Parasitic Nematodes in Annual Medics

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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 possible that parasitic nematodes associated with subterranean clover could pose a similar threat to annual medics. It would be

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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interesting to compare the incidence and severity of plant parasitic nematodes in annual medic pastures with those in subterranean clover pastures or other legumes grown in rotation.

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, the authors did not conclude that *Pratylenchus* was a key causal factor in the decline of annual medics pastures in that state. 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 ca 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.*, 1997; Hill and Korte, 1998) have also noted the potential importance of *Pratylenchus* in medic pastures. *Pratylenchus neglectus* and *P. thornei* have been recorded in medics (e.g., Taylor *et al.*, 2000), but 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 cultivars 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 cultivars, 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, pers comm.). 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.

### 3.5 Host Resistance

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The utilisation of resistant cultivars offers the most cost-effective, long-term control measure for annual pasture legume diseases, 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 *Kabatiella* on *Trifolium* spp. (Barbetti, 1996). These same opportunities exist for at least some diseases, such as Phoma blackstem disease, in annual medics (Barbetti, 1993a, 1995b) providing large scale screening of germplasm for resistance is undertaken as has been done for many years against *Kabatiella* in Australia (Nichols *et al.*, 1996).

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, 1990, 1995b) and elsewhere (Renfro and Sprague, 1959; O'Neill

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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*et al.* 2003). In Australia, useful resistance to post-emergence damping-off caused by *Pythium* spp. has been identified in *M. denticulata* (Andrew 1962). Elsewhere, useful levels of resistance against *Colletotrichum trifolii* (Raynal, 1977; Elgin and Ostazeski, 1982; Lamprecht and Knox-Davies, 1984c; Lamprecht, 1984c, 1986b; Troeung and Gosset, 1990; O'Neill and Bauchan, 2000), *Pseudopeziza medicaginis* (Schmiedeknecht, 1959), have been reported. *Phytophthora medicaginis* (Haan *et al.*, 2002) has only been reported in annual medics outside of Australia. While only low levels of resistance to *P. clandestina* has been reported to date 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 (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. This may be related the proportionately smaller acreages sown to annual medics compared with subterranean clover. 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 on annual medics as remains the case with subterranean clover.

Realistically, there is a need for commercial cultivars 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. For example, the severe losses from *Phoma medicaginis* in susceptible annual medics across southern Australia and the stimulation of coumestrol and other phyto-oestrogen production associated with this infection (Barbetti and Nichols, 1991b), emphasises the double value of future cultivars carrying resistance to this disease.

### 3.5.1 Individual sources of host resistance

Commercial species of annual medics in Australia are reliant upon the determination of sources of resistance against serious necrotrophic soil-borne pathogens. Cultivar development programs targeting disease resistance, such as those developed for Phoma blackstem disease in Australia (Barbetti, 1989a, 1990, 1993) and in the United States of America (O'Neill *et al.*, 2003), need to be expanded in Australia. Such sources of resistance could be identified in screening programs and can be useful both as parental materials in breeding and for release of resistant cultivars (Barbetti, 1995b). Only such incorporation of this resistance in commercial cultivars could offer a promising long-term management strategy for foliar disease 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 three accessions of *M. polymorpha* that had resistance to *Phytophthora medicaginis*. 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* that exhibited a high level of resistance to *Phytophthora medicaginis*. Similarly, O'Neill and Bauchan (2000) identified 14 accessions of the 201 tested, that had resistance to *Colletotrichum trifolii*, and this included accessions in *M. murex*, *M. muricoliptis*, *M. polymorpha* var. *brevispina*, *M. polymorpha* var. *polymorpha*, *M. radiata*, *M. soleirolii*, *M. truncatula* and *M. turbinata*. In the same way, in South Africa, Lamprecht (1986b) identified *M. truncatula* cultivars Borung and Cyfield as the most resistant of nine cultivars tested from across four annual medics (*viz.* *M. littoralis*, *M. tornata*, *M. truncatula* and *M. scutellata*).

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 pathogens. In Australia,

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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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 cultivars. 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*.

### 3.6 Cultural Control Strategies

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#### 3.6.1 Grazing

While the normal grazing of annual medic pastures has probably provided substantial disease control of foliar diseases it probably offers much less benefit towards control of soil-borne diseases. Outstanding examples of the value of grazing in relation to control of foliar diseases in other pasture legumes include annual *Trifolium* spp. in relation to *Kabatiella* (Beale, 1972; Bokor and Chatel, 1973; Bokor *et al.*, 1978; Clarke, 1980; Barbetti, 1996). For example, Anderson *et al.* (1982) found that high stocking rates of 10-12 sheep/ha reduced *Kabatiella* disease incidence in subterranean clover by 20-35% compared with 6 sheep/ha. Similar observations have been noted for diseases on annual medic pastures (Barbetti 1989a; M.J. 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 susceptible cultivars 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. However, grazing may damage plants and make them more susceptible to some soil- and trash-borne diseases, such as occurs with Phoma blackstem disease in Australia (MJ Barbetti, unpubl.) and as likely occurs for some root disorders (e.g., *Phytophthora clandestina* root rot) as occurs in other pasture legumes such as *Trifolium* spp. (Barbetti *et al.*, 1986). There is a need to understand the grazing thresholds to reduce stress/disease to acceptable levels and facilitate rapid plant recovery from root disease.

#### 3.6.2 Tillage and burning

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. Tillage associated with pasture renovation and burning can be helpful by removing infested residues, as shown for subterranean clover (Beale, 1976; Bokor *et al.*, 1978), even if such benefits are sometimes short lived due to rapid saprophytic build-up of the pathogen (Beale, 1976). If resistant types of annual medics replace susceptible cultivars, disease control through heavy grazing, burning or tillage will become relatively less important. 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 cultivars become available.

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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### 3.6.3 Nutrition

Mineral nutrients may either increase or decrease the tolerance or the resistance of the plants to pathogens and pests (Marschner, 2002). Nutrition is an important component of disease management, with all fundamental mineral elements reportedly influencing disease incidence or severity (Engelhard, 1989). 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 (Gilkes and Hunt, 1992) 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 (Engelhard, 1989), 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<sub>3</sub><sup>-</sup>, or Fe, and where plant tissues had relatively high levels of total N, K, and S, Cu, Zn, Mn, or NO<sub>3</sub> 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. In contrast to soil-borne pathogens, the interaction of nutrition appears to be less complex in relation to at least some foliar pathogens in pasture legumes, for example, the increased susceptibility of subterranean clover to *Cercospora zebrina* when K levels in soils are significantly below or above 'normal' (J. Dempster, M. You, M.J. Barbetti and K. Sivasithamparam, unpubl.). The full potential for benefits of reduced disease by manipulating nutrition is outlined more fully in Section 7 of this report.

### 3.6.4 Rotations

Annual *Medicago* spp. are utilised extensively as a rotational crop because they give a high yield of good quality forage and are tolerant of grazing (Chatterton and Chatterton, 1990). As a rotational crop, annual medics are utilised most widely with wheat, such as in Australia (Sadras *et al.*, 2004), South Africa (Lamprecht *et al.*, 1990), Tunisia (Breth, 1975), Syria (Nordblom *et al.*, 1994), the Near East (Chatterton and Chatterton, 1996), Chile (Poza *et al.*, 1989), Canada (Frazer *et al.*, 2004) the USA (Sheafer *et al.*, 2001) and Mexico (Fischer *et al.*, 2002). 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*s (e.g., AG8) can be reduced by soil disturbance (till deep, sow shallow), trash retention (suppression) and nutritional amendments such as Zn.

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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### 3.7 Fungicidal Control Strategies

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Fungicides are unlikely to provide effective or economic control of soil-borne diseases of annual medics. Disease control with fungicides will become less important if cultivars with improved resistance are deployed, but effective fungicides need to be available, along with appropriate cultural methods, if such resistance is overcome by changes in the pathogen populations. This is in contrast to foliar diseases, where 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). For example, up to 9,000 ha of subterranean clover were treated annually with fungicide in Western Australia alone for control of *Kabatiella* (Barbetti, 1996).

### 3.8 Integrated Disease Management

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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 cultivars and/or application of close grazing provides more benefit than the same or greater amounts of fungicides applied to susceptible cultivars, 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 necrotrophic fungal pathogens of annual medics. Integration of control strategies will remain a key focus until cultivars of annual medics with improved host resistance to parasitic nematodes and necrotrophic fungal 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.

### 3.9 Conclusion

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Although research on the impact on medics of necrotrophic fungal diseases and root-feeding nematodes of annual medics is relatively small compared to that of subterranean clover, it is likely to be significantly greater and more widespread than previously recognised. In addition, there are many instances where diseases on annual medics are common to other rotational crops. For example, *Phoma medicaginis* from annual medics and peas (*Pisum sativum*) can be cross-infected (Barbetti and Khan, 1987), resulting in pathogen survival in the absence of one host during crop rotation cycles and also reducing or negating the expected 'disease break' benefits from rotating crop species. Similarly, there are common disease pathogen susceptibility patterns of wheat and annual medic diseases such as those caused by some strains of *Rhizoctonia solani* (Sivasithamparam, 1993), *Pythium irregulare* (K Sivasithamparam, unpubl.) and the same applies for plant parasitic nematode species such as *Pratylenchus* spp. that occur across a range of different pasture and crop species. There are a significant number of 'diseases-in-common' between annual and perennial *Medicago* spp. and also between annual medics with other widely cultivated pasture legumes such as subterranean clover (Barbetti, 1989b), including those caused by species of *Pratylenchus*, *Pythium*, *Fusarium*, *Phoma*, *Rhizoctonia* and *Phytophthora* in relation to root diseases alone. In addition, there are the side effects resulting from production of phyto-

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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oestrogens and mycotoxins as a consequence of infection by one or more of these necrotrophic fungal pathogens. Such pathogens include *F. acuminatum*, *F. avenaceum*, and *F. graminearum*, associated with annual medics in Western Australia (Barbetti and Allen, 2005) that can readily cross-infect different pasture and crop host genera (Barbetti and Allen, 2006).

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 necrotrophic fungal pathogens and/or plant parasitic nematodes.

## 4 Soil-borne Diseases of Lucerne and Other New Legumes

### 4.1 Introduction

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Lucerne is a deep-rooted perennial forage legume with a potential important role in preventing dryland salinity in southern Australian cropping regions. Repeated cycles of cereal production have created a water-use imbalance, which is placing the industry under threat through rising saline water tables and resultant dryland salinity. Lucerne is being incorporated into cropping systems in an attempt to reduce groundwater recharge and improve the sustainability of grain production (Humphries and Auricht, 2001).

The agronomy of lucerne and new legumes in rainfed pastures of southern Australia remains to be explored further, especially in relation to the nature and spectrum of pathogens that will replace or add-on to those that are dominant in irrigated pastures. Lucerne pastures that provide a green bridge in the Mediterranean summer are likely to attract biotrophic pathogens and may benefit for reductions in inoculum build-up or carry-over that occurs with necrotrophic pathogens in Mediterranean environments (Sivasithamparam, 1993). Much of the results of work done on soil-borne diseases of lucerne, in the USA for instance, cannot be extrapolated to the rainfed environments of southern Australia.

In Australia, approximately 200,000 ha of lucerne are grown under irrigation for hay production, and another 3.5M ha are used in dry land farming operations (Pearson *et al.*, 1997). In particular, there is considerable potential for expansion of these dryland areas, with an additional estimated 86M ha and 9M ha suitable for planting in eastern and Western Australia, respectively (Hill, 1996). Lucerne is currently being promoted in Western Australia as one of the best options to reverse the hydrological imbalance resulting from growing annual crops and pastures (George *et al.*, 1997). The hydrological imbalance results in an increased amount of water draining below the root-zone of annual agricultural species relative to the previous native vegetation. Increased drainage has resulted in water tables rising, mobilization of salts stored in the soil, and consequently the spread of salinity (Hatton and Nulsen, 1999).

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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### 4.2 Soil-borne Pathogens and their Impact

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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, 1999; 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 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 New South Wales (Anon., 1975b). 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 low (Clarke, 1981b, 1999). 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 cultivars. 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 cultivars 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. 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 cultivars 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. More recently, Gramshaw *et al.* (1985) studied the field reaction on



## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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North American and Australian lucernes to *P. megasperma* and demonstrated that two Australian lucernes had resistance equal to that found in the best North American lucerne cultivars.

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, 1999). 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 plant refuse.

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, 1999). 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, 1999). Orange-red flecks can usually be seen just below the disease front. Eventually the root becomes necrotic and the plant dies. Control involves improving drainage and using 2-3 year rotations between crops (Clarke, 1981b).

*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 euteiches* was recorded as possible cause of poor lucerne establishment in Queensland and showed high levels of virulence on lucerne (Abbo and Irwin, 1990).

Charcoal rot (*Macrophomina phaseolina*) was detected in all lucerne varieties and in all Agzones in Western Australia (Wright and Jones, 2003).

Spring blackstem and leaf spot, caused *Phoma medicaginis*, is common on lucerne. In Queensland the disease is 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). Numerous small black to dark brown spots develop on the lower leaves, petioles and stems. The irregular shaped lesions on the leaves 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. 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). Severely infected stands should be cut as soon as possible to reduce leaf loss (Clarke, 1981a).

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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Anthrachnose, 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 New South Wales 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. Use of 2-3 year rotations and more resistant cultivars 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.

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. 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). 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. Control involves improving drainage and irrigation techniques and using resistant varieties (Clarke, 1981b).

Sclerotium wilt, caused by the fungus *Sclerotium rolfsii*, is more common in areas of high rainfall and high humidity at temperatures >25°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). Complete incorporation of organic matter residues into the soil prior to sowing new areas may help reduce losses in young plants (Anon., 1972).

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 New South Wales 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

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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particularly thrive in wet or waterlogged soils and are probably the most common pathogens involved in damping-off of lucerne in southern Australia. Control 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, 1971b).

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. He suggested that correction of unsatisfactory cultural conditions was the most practicable way of reducing losses from this 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 its occurrence in southern Australia or about the control of this disease where it does occur (Purss, 1965).

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. Root lesion nematodes are migratory endoparasites and they 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. (Shashi Sharma, unpubl. 2001).

Lucerne yellows disease is attributed to a phytoplasma (Fletcher, 1980, McGechan, 1980) is one of several major lucerne diseases in New South Wales (Stovold, 1983, McDonald *et al.*, 2003). The symptoms include discoloration of leaves ranging from yellow to red (Stovold, 1983) that affect the entire foliage (Pilkington *et al.*, 1999). Roots of affected plants have a characteristic yellow-brown discoloration immediately under the periderm of the taproot (Stovold, 1983, Pilkington *et al.*, 2002).

In Australia, 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 soilborne 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 cultivars released ([www.sardi.sa.gov.au/pages/pastures/breeding\\_lucerne.htm](http://www.sardi.sa.gov.au/pages/pastures/breeding_lucerne.htm)). The root-feeding nematodes, *Meloidogyne* and *Pratylenchus* spp., can also be damaging to lucerne (Stuteville and Erwin, 1990) and 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 limited research in Australia on parasitic nematodes of lucerne clearly needs to be redressed given the growing interest in using lucerne for its environmental benefits (e.g., Turner and Asseng, 2005).

## **5 Soil-borne Diseases of Grasses**

In 1987, Johnstone and Barbetti (1987) considered that research into the 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. Much of the little information 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 predominantly weedy species such as *Bromus* and *Hordeum*. Most of these are invariably susceptible to pathogens such as the 'take all' fungus (*Gaeumannomyces graminis* var. *tritici*) and *Rhizoctonia solani*. However, these 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, 1998b). 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. However, this maybe 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).

Wong (1975) described kikuyu yellows, a disease that seriously reduces the productivity of affected kikuyu stands in New South Wales. 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).

Fungal pathogens have been demonstrated to have significant impact on productivity of ryegrass. Dewan and Sivasithamparam (1988a) showed that *Pythium* spp. in particular are highly pathogenic to ryegrass and can cause significant damage to roots, as were species of *Trichoderma* (1988b). *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, 1988c). Glasshouse studies with cereal root pathogens have established the pathogenicity on ryegrass of the pathogen responsible for 'take all' disease, *Gaeumannomyces graminis* var. *tritici*, and that it can cause significant damage to ryegrass (Dewan and Sivasithamparam, 1988d). However, it is noteworthy that certain strains of *Gaeumannomyces 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 as it explained, for the first time, the dominance of ryegrass in 'take all' patches within wheat crops in the field in Western Australia.

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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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 per cent. 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 *Helminthosporium* 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 important in southern Australia. Resistance to *Helminthosporium* spp. and *Drechslera* spp. has been assessed by a Swedish worker (Braverman, 1986) who found that tetraploid cultivars were less infected than diploids, and that less winter hardy cultivars were more resistant.

Another area where soil-borne pathogens have been observed to cause problems on grasses is during establishment (Clarke and Eagling, 1994). Overseas, Lewis (1988) reported that *Fusarium culmorum* as an important soil-borne pathogen of perennial ryegrass and reported it was isolated from ungerminated seeds and dead seedlings. Lewis reported that fungicide seed treatment with benomyl and captan increased herbage yields. However, he reported that this fungicide treatment alone only increased seedling emergence at 1 of the 5 sowings, and that improved seedling vigour may have played a role in reducing attacks from soil borne pathogenic fungi (Lewis, 1988). 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.).

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).

Clarke and Eagling (1994), reported an alarming trend towards the reduction of pasture plant pathology expertise throughout Australasia and that trend needs to be reversed to allow adequate resourcing of breeding programmes and to ensure the ability to effectively respond to the introduction of any exotic grass pathogens. We have seen nothing to suggest that this trend has been altered in any way in the 12 years following their review.

The importance of root nematodes in Australian pasture grasses has not been investigated. The role of grass weeds and grasses of self-regenerating pastures in the cropping zone in maintaining *Pratylenchus* populations, problematic pests of crops, was 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 to be verified across a much wider range of agro-ecosystems.

## **6 Need to Improve Assessment of the Impacts of Soil-borne Diseases**

### **6.1 Introduction**

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Diseases not only cause important losses in pasture and forage species and as a consequence decrease the quality and quantity of the feedbase for animal production, but also affect crops grown in rotation with pastures. The extent to which these losses in legume species can adversely affect agricultural industries such as wool, meat, dairy, cereals, oilseeds and pasture seeds has been reviewed (e.g. Graham *et al.*, 1979; Haggard *et al.*, 1984; Barnett and Diachun, 1985; Raynal *et al.*, 1989; Braverman *et al.*, 1986; Campbell, 1986; Edwardson and Christie, 1986; Johnstone and Barbetti, 1987; Cook and Yeates, 1993; Lenné, 1994a, 1994b). In contrast, 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. However, even when there is available information on losses from soil-borne pathogens, much of the published information comes from experiments not representative or only partially representative of commercial pastures, particularly grazed ones containing a mixture of plant species. There is a need to assess losses in ways and situations such that the data obtained can be confidently used to make rational disease management and economic assessments.

Pathogen-induced losses can be direct or indirect. For the purposes of this report direct losses include diminished plant growth (i.e. decreased herbage), nutritional value (e.g., protein, specific amino acid and water soluble carbohydrate content, and dry matter digestibility), palatability, seed set and seed viability, and increased production of detrimental metabolites (e.g., phyto-oestrogens, tannins, phenols, mycotoxins). Indirect losses include undesirable changes in pasture composition, less residual fixed nitrogen, diminished utilisation of inputs to plant growth (e.g., inefficient fertiliser and water use) and animal productivity, and increased cost and side-effects of control.

It is not simple to quantify losses in a meaningful way. Since pastures are usually heterogeneous, consisting of more than one plant species, assessment of yield losses is more difficult than in arable crops (Cook and Yeates, 1993). There are many problems in obtaining meaningful data on pathogen-induced losses in pastures. The study of the races of *Phytophthora clandestina* should present an ideal case study to quantify losses from root disease in a more meaningful way.

### **6.2 Assessing Costs and Benefits of Controlling Disease-induced Losses**

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Bodies funding research on pasture diseases and representatives from affected industries (e.g., wool, meat, dairy, cereals, oilseeds and pasture seeds) increasingly demand more reliable data on disease-induced losses and the potential financial benefits of disease control before providing research funding. As a consequence, an increased amount of survey and disease-induced biological yield loss assessment was for a time undertaken by researchers involved in pasture and forage research. Although there was some earlier work on pasture disease losses (e.g., Hutton and Peake, 1954; Walker, 1956), before 1970 many pasture researchers did not perceive diseases to be important enough to justify research (Anon., 1988). Although this perception is now gradually changing, adequate research funds for pasture research are increasingly difficult to obtain in

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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Australia. A clear definition of commercial importance is essential before funding based on levies on the industries or from government is provided.

Demonstration of how widespread a pathogen is and what losses it results in are both required before economic analysis of the overall losses caused and the financial benefits likely to arise from disease control can be made. However, knowledge in this area is still deficient for many soil-borne pathogens of annual legumes such as subterranean clover, for most soil-borne pathogens of medics, lucerne and, in particular, for soil-borne pathogens of grasses. The biological and economic complexity of pastures as part of a larger system has contributed to this deficiency. This complexity arises because, apart from their direct effect on the pasture seeds industry, pastures have an indirect output *via* animal and crop production, and because the influence of disease has to be considered along with the many other factors affecting their yield potential. The value of pastures change with time because the profitability of the industries which they support are set by factors such as the market price of the animals and their products, the availability of alternative feed, the market price of nitrogen fertiliser, grain, etc. Their value is also influenced by seasonal conditions, pastures being worth more when feed is scarce, such as during periods of drought. Herbage loss estimates need to be set against periods of low feed availability (e.g., autumn to early winter in southern Australia), while seed losses in annual pastures need to be set against the early season regeneration phase when feed is limiting and also set against the cost of reseedling.

Perceptions about pasture diseases and their relative importance are often misleading. For example, Lenné (1989) suggested that, if animal production is not decreased by pasture disease because factors such as low stocking rate and poor pasture utilisation enable farmers to absorb the losses caused, then disease is not a serious problem irrespective of its severity because overall animal production is unaffected. We do not agree with this approach as it ignores the potential for increase in profit from increased carrying capacity and available fodder due to disease control. When fixed costs remain the same, the extra feed resulting from overcoming the disease problem can have a dramatic impact on profit. If the potential for increased profits is there, the fact that the farmer does not exploit it does not mean that it does not exist. Also, when assessing the benefits of pathogen control, often the only comparison made is between the value of the direct biological yield loss and the direct cost of the control treatments and their implementation. Factors such as the contribution to crops grown in rotation (e.g., *via* nitrogen build up, providing a 'disease break' from fungal cereal diseases, and improving soil structure) are often excluded from assessments of the relative benefits. When these other factors and the additional costs of replanting and re-establishing deteriorated pastures are also taken into consideration, then the total losses from particular pathogens are likely to be far greater than currently perceived. This may be even more so if pasture deterioration as a result of disease has led to erosion problems. Economic evaluation and modelling is being more widely used in determining the financial significance of disease-induced losses in pastures and the benefits due to disease control, but its validity depends on the assumptions made. When these assumptions are based on sound survey and yield loss data obtained over several years, predictions of the benefits arising from controlling pasture diseases under different scenarios can be very useful to the industry concerned. Examples of packages that could be used include the Research Evaluation Spreadsheet developed by Coelli and Young (1994), and the Western Australian Dairy Farm Model developed by Olney and Kirk (1989). There would be benefit from economic evaluation packages and models that include the full array of direct and indirect losses, and if possible animal production losses, being improved and made more user-friendly and applied more widely. The direct and indirect losses from pathogens affect not just the individual producers, but extend to rural communities, exporters, and consumers. These downstream effects are seldom if ever taken into account when assessing the impact of disease-induced losses on pastures.

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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### 6.3 Where to Next in Relation to Improving Assessment of the Impacts of Soil-borne Diseases

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In relation to assessment of the impacts of soil-borne diseases, there is a paucity of information on these impacts for medic, lucerne and grass diseases across southern Australia. In the first instance, there would be benefit from knowing where soil-borne pathogens annual medic, perennial lucerne, and of annual and perennial grasses occur at high incidences in southern Australia. Then sensible yield loss predictions should be made based on grazed swards, if possible mixed species or, better still, the plots should be marked out in commercial pastures at a wide range of sites across southern Australia. Such impact assessments, ideally, will include assessment of numbers of plants regenerating herbage and seed yield losses, along with changes in botanical composition, persistence after the first year, effects of soil-borne diseases on yield quality (such as increased toxin production and changes in nutritional value), and importantly, also include factors such as contribution to subsequent cereal crops (e.g., via nitrogen fixation, providing a 'disease break', and improving soil structure), in such assessments.

## 7 Interaction of Nutrition and Soil-borne Diseases

### 7.1 Introduction

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Excellent comprehensive reviews exist on the effects of plant nutrition on the development of diseases (Sadasivan, 1965; Huber, 1980; Graham, 1983; Marschner, 1986; Engelhard, 1989). This section therefore has been limited to the consideration of the effects of soil nutrients on the conduciveness of soils to soil borne fungal plant pathogens. 'Soil nutrients', in the context of discussion of this section, are minerals and organic molecules available in or added as amendments to soil. Plants take, up inorganic nutrients while micro-organisms, including pathogens and antagonists may utilize both inorganic and organic nutrients.

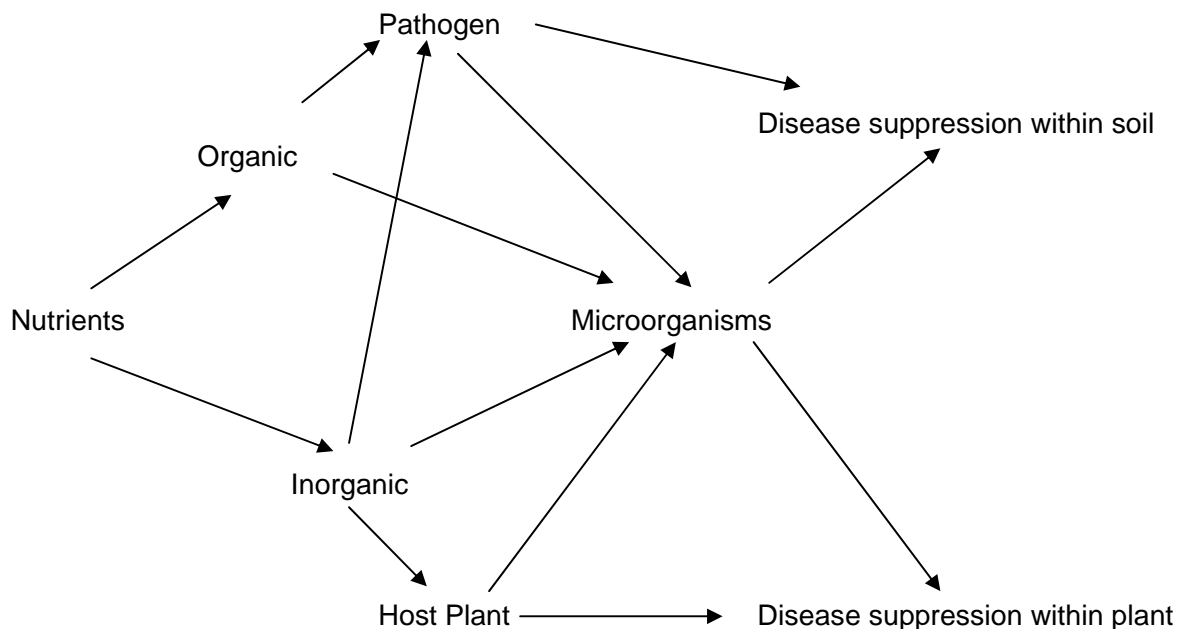
Nutritional deficiencies such as those which occur in the old, leached soils of southern Australia reduce the natural resistance of crop plants to disease (Graham, 1983). Although it may be possible to determine the role of host nutrition in disease development by testing for pathogenicity of a causal organism at various points along the nutrient response curve of the host, the involvement of soil microbiota in such disease amelioration cannot be discounted unless the tests are also conducted in the absence of associated soil microflora. It is however generally accepted that diseases, especially those caused by soil-borne necrotrophs are frequently exacerbated across much of southern Australia by nutritional stress imposed on the plant hosts. Correction of these deficiencies in most cases reduces the severity rather than the incidence of diseases, but still offers some opportunities for exploitation.

Soil fertility can affect disease development by its effect on the activities of soil microflora which are inhibitory to the soil-borne pathogens. Components of soil fertility have been associated with naturally occurring soil suppressiveness (Cook and Baker, 1983; Schneider, 1982; Mukerji and Garg, 1988). For instance, a range of factors including organic matter,  $\text{Ca}^{2+}$ ,  $\text{NH}_4^+$ , water content, soil pH and extracts from the rhizosphere of certain plants influence the suppressiveness of certain soils to *Phytophthora* (Cook and Baker, 1983).



## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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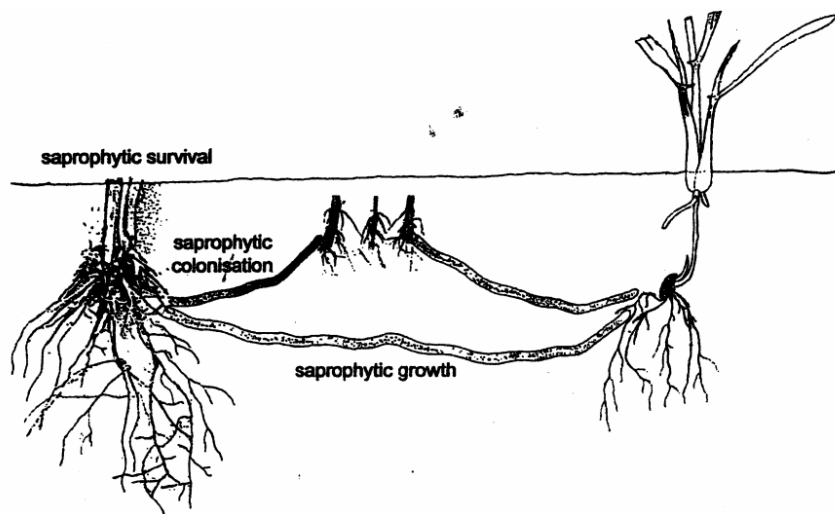
**Figure 6.** The ways by which soil nutrients can affect diseases by direct effects on the pathogen or by their effects on the nutrition of the plant host or through their influence on microbial antagonist(s) (K. Sivasithamparam 1997).

As illustrated in Fig. 6 above, soil nutrients can affect diseases by direct effects on the pathogen or by their effects on the nutrition of the plant host or through their influence on the microbial antagonist(s). To understand the relationships between soil nutrients and soil suppressiveness, it would be useful to relate all the effects in terms of 'pathogen suppression' or 'disease suppression'. 'Pathogen suppression', for the purposes of this report, is considered as the microbial suppression of the pathogen outside and without the direct involvement of the plant host. In contrast, 'disease suppression' occurs within the host, commonly with host involvement (Cook and Baker, 1983; Hornby, 1983; Simon and Sivasithamparam, 1989). Both forms of suppression are discussed in this section of the report.

# Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

## 7.2 Nutrient Effects on 'Pathogen Suppression'

'Pathogen suppression' may be exerted at any one or combinations of the saprophytic phases expounded by Garrett (1956), viz. (a) saprophytic survival (b) saprophytic growth or (c) saprophytic colonization (Fig. 7). Clearly, nutrients may have direct or indirect effects on the microorganisms which inhibit or stimulate the pathogen at these phases of saprophytism.



**Figure 7.** Aspects of soil saprophytism relevant to both the pathogens and the biocontrol agents. Left to right: saprophytic survival involving the survival of the pathogen in residues of tissues colonized during its parasitic phase; saprophytic colonization of soil organic matter by the pathogen preceding parasitic activity in the cropping season; saprophytic growth of the mycelium of the pathogen through soil, from the substrate where it survived to the young root (Based on Garrett, 1970, as presented by K. Sithamparam 2002)

### 7.2.1 Saprophytic survival: dormancy

Although physiological activity in a dormant spore is minimal, microbial forces do play a significant role in their fate especially through fungistasis (inhibition of germination of a fungal propagule in soil) (Lockwood, 1990). Negation of fungistasis through supply of nutrients emphasizes the role of microbial competition for nutrients in the fate of the propagules of not only of soil-borne pathogens but those of antagonists as well. Such fungistasis is considered to be the basis for the preference of inoculum based on mycelial states rather than spores when using antagonists such as *Trichoderma* spp. (Chet, 1987; Papavizas, 1985).

Nutrients are important in the establishment of hyperparasitism. For example, susceptibility of sclerotia of *Sclerotium rolfsii* to microbial attack following shock treatments such as drying and exposure to chemicals are considered to result from the release of nutrients from sclerotia which are then subjected to competition from the antagonists and the sclerotium itself (Henis, 1984). This hypothesis corroborates the observation that neither emerging mycelia nor germinating sclerotia of

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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*Sclerotium rolfsii*, a pathogen of lucerne, are attacked by *Trichoderma* in soil. Nutrient status of the biological control agent (BCA) inoculum is critical in all instances where pathogen suppression could be contemplated for southern Australia. In the cases of obligate mycoparasites such as *Sporidesmium sclerotivorum*, the energy available in spores is critical until infection is established (Adams *et al.*, 1985). Success in the destruction of dormant propagules through introduced BCAs has been recorded mainly with fungal sclerotia. It is noteworthy that in the case of *Sclerotinia* spp., including *S. trifoliorum* and *S. sclerotiorum* on pasture legumes, most of the common facultative mycoparasites were successful as BCAs only when the spores were introduced on the surface of the sclerotia, while only the obligate species such as *Sporidesmium sclerotivorum* were capable of causing extensive destruction when applied as a soil infestation (Adams, 1989). Predation of dormant fungal propagules by mycophagous amoebae are not uncommon (Old and Darbyshire, 1978), but little is known of the nutritional behaviour of amoebae mainly because they are not at present culturable. There is no relevant information on the potential to utilize mycophagous amoebae for control of soil-borne pathogens in pastures for southern Australia.

### 7.2.2 Saprophytic survival: growth on substrate – on crop residues for both legumes and grasses

The pathogen that causes 'take all' disease in wheat and also attacks some pasture grasses, *Gaeumannomyces graminis* var. *tritici*, survives in southern Australia by growth, albeit slow, in the substrate they colonize during their parasitic phase. Garrett's (1956) work on this pathogen showed that nitrogen (N) which may be immobilized by soil microorganisms can be limiting for pathogen growth within crop residues. Garrett (1985) more recently emphasized the role of soil microorganisms in this saprophytic survival.

Garrett's observations on the effect of soil N on 'take all' has been confirmed in other studies including those on field inoculum of the 'take all' fungus (Cotterill and Sivasithamparam, 1988a,b). Saprophytic growth in residues is not common in environments such as southern Australia and, in particular, the Mediterranean-type environment of the wheat-belt of Western Australia, where the period between cropping seasons is hot and dry and little or no growth of the pathogen or the antagonist occurs.

### 7.2.3 Saprophytic growth in soil

Several soil-borne plant pathogenic fungi such as *Rhizoctonia solani* and *Gaeumannomyces graminis* var. *tritici* grow well in soils of southern Australia. Their growth extensions may become more sensitive to microbial antagonism as the thallus leaves its food base. This has been shown with the 'take all' fungus in nutrient poor Western Australian soil (Simon *et al.*, 1987). It is likely that the hyphosphere in such nutrient poor soils could be a nutritionally attractive niche for microbes. Indeed the release of nutrients by hyphae has been proposed as the basis for the attraction of mycoparasites to hyphae of plant pathogenic fungi by Foley and Deacon (1986). They suggested that the resistance to parasitism by the root rot causing pathogen *Pythium* spp. seems to depend, at least partly, on the low levels of nutrients leaking from host hyphae. Such leakage from pathogens could be induced by antagonists. Lewis *et al.* (1991) showed that *Gliocladium virens* induced cytoplasmic leakage from *Rhizoctonia solani*.

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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Simon and Sivasithamparam (1990) found that the drop in soil pH after fertilization with ammoniacal nitrogen resulted in dominance of *Trichoderma koningii* in the fertilized soil. The same soil-borne species also inhibited the growth of the 'take all' fungus in soil. Liming of the soil reduced the activity of the antagonist and increased saprophytic growth of the pathogen and subsequent development of the 'take all' disease in the crop. Glenn and Sivasithamparam (1991) felt it unlikely that the 'take all' disease response to soil pH resulted from direct effects on the saprophytic phase of the fungus. They stated that the increase in soil  $\text{Ca}^{2+}$  was not integral to the increased growth of the fungus and suggested other effects such as a pH influence on nutrient availability and altered activities of antagonists, *i.e.*, *Trichoderma* (Simon and Sivasithamparam, 1990) or pseudomonads (Smiley, 1978a, b). Clearly, there is potential to identify and utilize antagonists to reduce saprophytic growth of pathogens in soils of southern Australia.

### 7.2.4 Saprophytic colonization

Pathogens such as *R. solani* are capable of extensive spread in soil by utilizing the available organic debris for energy (Garrett, 1963). This has been aptly termed 'combative migration' (Cooke and Rayner, 1984) whereby colonization occurs in the presence and often in competition with other soil microorganisms. Organic substrate may already be partly 'occupied' (Bruehl, 1987). In the soils of southern Australia which are nutrient poor and low in microbial activity, even pathogens such as the 'take all' fungus, which has a low saprophytic ability (Garrett, 1963), can succeed in combative migration (Glenn *et al.*, 1988). This certainly indicates that reduced inoculum potential of certain soil-borne pathogens during the saprophytic colonization phase is possible by manipulation of the nutrient and microbial status of soils supporting pastures across southern Australia.

### 7.2.5. General suppression (sensu Cook and Baker)

According to Cook and Baker (1983), general suppression of a pathogen is "directly related to the total amount of microbiological activity at a time critical to the pathogen". Suppression is related to the fertility of a soil or growth medium. Addition of carbon with nitrogen stimulates different types of antagonisms (competition, antibiosis, lysis, parasitism) more so than addition of carbon alone (Mangenot and Diem, 1979). This is likely to occur in pastures with a balanced legume content. A nutrient which is favourable to the pathogen under axenic (non competitive conditions) conditions however may prove to be unfavourable under natural conditions if it also stimulates potential antagonists.

The control of *Phytophthora* (Cook and Baker, 1983) by introducing organic C (Chen *et al.*, 1988, Hardy and Sivasithamparam, 1991) are examples of general suppression in which competition occurs between soil-borne pathogen and the background microflora for nutrients. As Alabouvette (1986) pointed out, 'general suppression' may operate hand in hand with 'specific suppression' and thus the two phenomena need not be mutually exclusive. Treatments that increased microbial activities in soil should be detrimental to the pathogens. According to Cook and Baker (1983), the larger the active microbial biomass of soil, the greater the sink for carbon, nitrogen, and energy in the soil and the less the likelihood that a finite supply of any one essential nutrient factor from a host will be adequate to satisfy the needs of the pathogen. However, C and/or N amendments may not be sufficient on their own to render a soil suppressive to a pathogen if another nutrient is limiting. For example, Ko and Kao (1989) suggested a combination of high Ca content and a large microbial

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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population as the cause for suppression of *Pythium* in the soil. They observed that soils high in Ca but low in microbial populations became suppressive when microbial populations were increased.

Single nutrient application to improve fertility can also result in microbial suppression of pathogens. Punja *et al.* (1986) found that one or two applications of  $\text{NH}_4\text{HCO}_3$ , (84 kg/ha N) reduced viability of *Sclerotium rolfsii* sclerotial survival structures. Enhanced leakage of nutrients from these fungal structures exposed to  $\text{NH}_4^+$ -N compounds in soil (Chun and Lockwood, 1985) and increased antagonism from soil microorganisms have been suggested as causes for the decline in inoculum populations (Punja, 1989), and such effects may apply to a wider range of soil-borne pathogens, including those affecting pasture species in southern Australia.

Fertilizers may also promote the activity of specific antagonists in soil. Fertilization with K increases whereas N decreases populations of *Penicillium funiculosum* (Kaufmann and Williams, 1965) which is antagonistic to *Fusarium* spp. and *R. solani* (Kaufmann and Williams, 1965) and *Phytophthora cinnamomi* (Tsao *et al.*, 1991). The potential to utilize fertilizer applications to increase antagonistic organisms to soil-borne pathogens of pasture species across southern Australia warrants further investigation.

### 7.3 Nutrient Effects on 'Disease Suppression'

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The effects soil nutrients have on disease development on hosts are generally indirect (as shown in Fig. 6). Nutrients might reduce disease by enhancing host resistance (induced host resistance) or by augmenting host receptivity of organisms involved in the suppression of disease. Nutrients can also directly affect the pathogen or antagonist.

#### 7.3.1 Direct effects on host: enhancement of resistance

Plant nutrients can reduce the severity of diseases through enhancement of plant growth and disease tolerance. The consequence is better effectiveness of the bio control agents because the disease is not so pervasive. Such a combination would be an ideal example of integrated biological control. Phosphates enhance host resistance by stimulating the production of phytoalexins (compounds produced by resistant plants challenged by the pathogen) (Gottstein and Kuc, 1989). Exposure to phytotoxic levels of  $\text{Cu}^{+2}$  (Bell, 1967) or to  $\text{NH}_4^+$  fertilizers (Chan and Wilhelm, 1984) can elicit production of phytoalexins. The control of oomycete pathogens by phosphoric acid is now recognized as host mediated (Cohen and Coffey, 1986) and not by the activity of rhizosphere or soil microbes (Wongwathanarat and Sivasithamparam, 1991), and this chemical is very successful in controlling root rot of subterranean clover caused by *Phytophthora clandestina* occurring in subterranean clover pastures throughout southern Australia (Greenhalgh *et al.*, 1994; M.J. Barbetti, unpubl.).

Recently (Homma and Arimoto, 1991), proposed that amendments of soil with nutrients such as potassium bicarbonate (1000 ppm), sodium bicarbonate (2000 ppm) or copper sulphate (160 ppm) could be used for the 'phytomineralotherapy' of plant diseases. These compounds are considered to have a curative as well as a nutritive role in plant health. Chitins and chitosans, known to be suitable substrates for actionomycete antagonists (Cook and Baker, 1983), have recently been reported to induce resistance of plants to fungal infections (Skjak Braek *et al.*, 1989).

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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Macro and micronutrient status of the plant can affect disease severity (Sadasivan, 1965; Graham, 1983). Improved plant nutrition is thought to increase tolerance and to alter or enhance specific host plant resistance mechanisms. For example, the increased severity of the 'take all' fungus in copper deficient plants (Wood and Robson, 1984) may be related to the decreased synthesis of lignin and consequent reduction in the build up of phenols (Robson *et al.*, 1981). Manganese deficiency was shown to decrease levels of phenols and lignins in wheat roots (Brown *et al.*, 1984). Indeed, many of the plant nutrients affect disease only in the deficiency range. Supra optimal amounts of nutrients do not provide further protection and may be detrimental in the case of nitrogen (Graham, 1983). Specific nutrients may have different effects on different pathogens on the same host (Huber, 1980; Engelhard 1989) and even in the same soil (Srihuttagam and Sivasithamparam, 1991). Forms rather than the element itself may influence disease. For example in the case of 'take all', the nitrate and ammoniacal forms of N can increase and decrease, respectively the expression of disease, all effects being mediated by soil microorganisms (Huber, 1980; Smiley, 1975). Smiley and Cook (1973) proposed that the suppression of 'take all' by ammonium nitrogen is largely a pH effect. When the changes in pH were prevented (with acid or base additives) the differences in 'take all' caused by ammonium and nitrate nitrogen were not evident. As pointed out earlier, this shift in pH could affect availability of nutrients (Cook, 1981) such as Mn or the activity of antagonistic microorganisms such as the fluorescent pseudomonads (Smiley, 1978 a, 1978b) and *Trichoderma* spp. (Simon and Sivasithamparam, 1989).

Manganese has received much attention in the past (Huber and Wilhelm, 1988; Graham, 1983) and is suspected not only of reducing disease by direct action on the inoculum of the pathogen, but also indirectly by acting on the physiology of the plant through its role in lignin synthesis (Graham 1983). Indeed, both mechanisms may be operational with soil applied manganese sulphate which decreases 'take all' in field grown wheat (Wilhelm *et al.*, 1988).

Mycorrhizal (Marx, 1972) and non mycorrhizal (Dewan and Sivasithamparam, 1988, 1989a, 1989b, 1989c, 1990, 1991; Gillespie Sasse *et al.*, 1991) cortical invading fungi can confer protection in host roots from root infecting pathogenic fungi. Some pathogenic fungi are inhibited by the production of antifungal compounds in ectomycorrhizal root systems (Krupa and Nylund, 1972; Krupa *et al.*, 1973). Nutrients which retard the activity of mycorrhizal fungi are likely to reduce this protective effect. For example, Bigg (1981) found that nitrate could inhibit mycorrhiza formation.

It is evident that little is currently known about the interaction of nutrition with the major soil-borne pathogens of most if not all pasture species important to southern Australia. There is significant potential for improved management of major soil-borne pathogens of pasture species across southern Australia from a better understanding the full potential for manipulating nutrition for improved disease control. Although most work with nutrition/disease interactions has been done with crop plants, it is expected that similar interactions would occur with pasture legumes and grasses.

## 8 Implications for Industry

The role played by necrotrophic soil-borne fungal and nematode diseases may in fact be far wider and have greater impact than previously considered. There are many instances where diseases on annual subterranean clover, medics, lucerne and grasses are common to other rotational crops. However, some pathogens, such as *Phoma medicaginis* from annual *Medicago* spp. and peas (*Pisum sativum*), can cross-infect (Barbetti and Khan, 1987), both to perpetuate the pathogen

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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survival in the absence of one host during crop rotation cycles and to reduce or even deprive the expected disease-break benefits from rotating crop species. Similarly, there are common disease pathogen susceptibility patterns of wheat and annual medic diseases such as some strains of *Rhizoctonia solani* and *Pythium irregulare* (Sivasithamparam, 1993). There are a significant number of diseases in-common between annual and perennial *Medicago* spp. and also between annual medics with other widely cultivated pasture legumes such as subterranean clover (Barbetti, 1989b), such as those caused by *Pythium*, *Fusarium*, *Phoma*, *Rhizoctonia* and *Phytophthora* species. In contrast, studies by Harvey *et al.* (2001) showed that host-mediated selection occurs in relation to cereal, medic and subterranean clover isolates of *Pythium irregulare*, suggested that there could even be some potential to somewhat reduce the impact of such pathogens that have a wide host range by manipulation of rotational practices. There are the side effects resulting from production of phyto-oestrogens and mycotoxins as a consequence of infection by one or more of these necrotrophic soil-borne fungal pathogens. Such pathogens include *F. acuminatum*, *F. avenaceum*, and *F. graminearum*, associated with annual medics in Western Australia (Barbetti and Allen, 2005, 2006) that can readily cross-infect different pasture (*e.g.*, subterranean clover and lucerne) and crop (*e.g.*, lupin, pea, chickpea, lentil) host genera.

### 8.1 Soil-borne Pathogens – Lack of Key Information on Impacts

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While there is frequently a lack of key information on disease impacts on productivity of annual pasture legume species (Barbetti *et al.*, 1996), it is for annual medics, lucerne and particularly grasses that this lack of information is most evident. The level and impact for each of the pathogens involved in poor productivity of these pasture species needs to be defined. Similar approaches have been made for subterranean clover (*e.g.*, Barbetti *et al.*, 1996) and the issues needing to be addressed for other pasture species would be similar. For example, disease impacts for these other pasture species need to be assessed throughout their geographic range, within and between years, and include periods when animal feed is limiting. A first step could be to introduce the appropriate disease into trials or find locations where the diseases occur naturally, as disease needs to occur as a forerunner to measuring effects of pathogens. In many cases this will involve having to inoculate rather than relying on natural spread. Different fungal infection levels can be established, as has been done for a range of different forage legumes, by choosing naturally infected, untreated plots and treating some with fungicides (*eg.* Sonoda, 1980; Cardozo *et al.*, 1983; Broscious *et al.*, 1987; Barbetti, 1987a, 1987b, 1992; Campbell and Duthrie, 1990; Greenhalgh *et al.*, 1994); using cultivars of varying resistance (*e.g.*, Devine and McMurtrey, 1975); or by using cultural management practices (*e.g.*, cultivation, fertilizer application and time of sowing) to generate different infection levels (*e.g.*, Barbetti, 1991).

While herbage and seed yield losses have largely been quantified for subterranean clover, for most necrotrophic soil-borne fungal pathogens of other pasture species, herbage and seed yield losses are the first parameter that still needs to be assessed, preferably in mixed swards and grazed pastures. Measurement of losses in commercial pastures, under normal grazing, provides the most relevant information, but is the hardest to achieve experimentally and so is rarely utilised, even for other annual forage legumes. Losses from disease in uncut/ungrazed monocultures are often larger than those in mown monocultures, grazed monocultures, or first year or regenerated mixed swards, or commercial pastures. For example, fungal diseases of annual medic caused up to 32% loss of medic herbage in un-grazed monoculture swards (Barbetti and Fang, 1991) but no more than 19% loss in medic herbage in a regenerated grazed situation (MJ Barbetti and DA Nicholas, unpubl.).

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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Seed yield loss can involve decreased seed numbers and size, and both components should be measured as has been done for *Phoma* blackstem on annual medics (Barbetti and Fang, 1991). Additionally, it can also include losses in germinability and subsequent losses incurred as a result of seedborne pathogen survival and transmission. However, frequently, only overall seed yield is measured, for either annual medics (Barbetti and Nichols, 1991b) or for other annual legume spp. (e.g., Barbetti, 1987a,b; Barbetti and Nichols, 1991a). Also, unless the amount of dormant seed of the pasture species in question in the seed bank is taken into account and deleted (e.g., Barbetti, 1987a), losses in seed yields can be significantly underestimated (e.g., Barbetti, 1991).

Soil-borne pathogens also affect numbers of plants regenerating and stand persistence after the first year. The extent of regeneration of swards reflects seed yield losses occurring in the previous season. The decrease in germination due to disease in the previous season can be assessed by making core or quadrat counts of newly-germinated seedlings to determine plant densities, as done for subterranean clover by Barbetti (1991) and, ideally, this needs to be done for each of the component species. Disease losses in legumes in annual pastures, when compounded year by year, can be sufficient to reduce legume seed reserves to below the levels needed to maintain legume dominance. The greater the effect on seed production the greater the reduction in productivity and persistence of susceptible legumes, leading eventually to severely deteriorated (weedy, low nitrogen fertility) pastures that need to be re-seeded. Similarly, regeneration and productivity can be affected by disease-induced reductions in seed size (e.g., Jones and Nicholas, 1992). Small seeds produce weak and uncompetitive seedlings, decreasing plant populations which eventually results in decreased yields (Black, 1956). Similar effects and reduced seed viability may occur as a result of production of diseased seed.

Little or no published information exists on the impact of pasture disease on animal productivity. This is not surprising, given the complexity of using animals to measure losses resulting from diseases on annual pastures legume species.

Beneficial effects (such as nitrogen availability) of subterranean clover, annual medics and lucerne on broad-acre crops can be negated by soil-borne pathogen that interfere with benefits (e.g., fixed nitrogen) that flow to the crops, such as cereals or canola. Annual medic, lucerne or other legume pastures can decrease carry-over of cereal diseases, but this rotation flexibility is lost if disease affects legume productivity to the extent that the grass component susceptible to the cereal pathogens significantly increases (Pottinger *et al.*, 1993). However, some pathogen carryover from pasture legumes can be a direct threat to rotational crops, such as both *R. solani* on annual pasture legumes that can cause losses in subsequent cereal and legume crops grown in the same field (Sweetingham 1990; Sivasithamparam, 1993).

There are several factors associated with several necrotrophic pathogens on one or more pasture species (and possibly annual medics in particular) that can affect quality (such as phyto-oestrogens and mycotoxins), and these also need to be considered. Other direct effects resulting from infection of subterranean clover and/or annual medics and lucerne include increases in the concentration of phyto-oestrogens, tannins, phenols and mycotoxins (also for grasses) and decreases in nutritive value from lower concentrations of protein, specific amino acids and water soluble carbohydrates, lower dry matter digestibility and reduced palatability for all pasture species (Barnett and Diachun, 1985; Lenné, 1989; Pottinger *et al.*, 1993). Poor feed quality, digestive upsets from grazing diseased herbage, diminished reproduction from ingestion of pathogen stimulated oestrogenic



## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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compounds, and under utilisation of feed due to avoidance of poor quality foliage by grazing animals, all will contribute to losses in animal productivity (e.g., Collins and Cox, 1984). Interactions between factors affecting quality must also be considered, for example, the *Phoma* found on annual medics can itself produce the mycotoxin brefeldin A (Mortimer *et al.*, 1977).

Evaluation of disease impacts could be improved made more user-friendly for use with determining disease impacts on pasture species. For example, Broschius *et al.* (1987) not only developed and compared yield loss functions for the complex of fungi that cause foliar disease in lucerne, but also evaluated the accuracy and precision of models. Earlier quantitative yield loss models for individual foliar fungal pathogens of lucerne (e.g., Berkenkamp, 1971; Basu, 1976) had attempted to attribute losses to individual pathogens despite the fact that foliar pathogens of annual medics occur as complexes and cause symptoms that often are not diagnostic for the organisms involved, as demonstrated for lucerne by Renfro and Kernkamp (1963). Clearly, demonstration of how widespread a pathogen is and what losses it causes are both required before economic analysis of the overall losses caused by soil-borne pathogens can be made. However, knowledge in this area is still deficient for all nematodes and for the majority of necrotrophic soil-borne pathogens of annual medics, lucerne and grasses in southern Australia. The biological and economic complexity of pastures as part of a larger system has contributed to this deficiency and, in particular, as a consequence of the fact that these pastures have an indirect output *via* animal and crop production. The value of all annual and perennial pasture species change with time because the profitability of the industries which they support are set by factors such as the market price of the animals and their products, the availability of alternative feed and the market price of artificial nitrogen fertiliser relative to costs associated with N from N-fixation (for subterranean clover, lucerne and annual medics). Their value is also influenced by seasonal conditions, pastures being worth more when feed is scarce, such as during periods of drought. Herbage loss estimates need to be set against periods of low feed availability (e.g., autumn to early winter across southern Australia). Unfortunately, factors that contribute to crops grown in rotation, such as nitrogen accumulation, cereal 'disease breaks' and improved soil structure, are often excluded from assessments of the relative benefits. When these other factors and the additional costs of replanting and restoring deteriorated pastures are also taken into consideration, then the total economic losses from necrotrophic soil-borne fungal pathogens and plant parasitic nematodes on pastures across southern Australia is likely far more significant than previously perceived.

It is noteworthy that the Mediterranean region has proved to be a productive source for collecting host germplasm with excellent resistance to one or more foliar and soil-borne necrotrophic pathogens. The value of this region as a source of resistance is highlighted by the example of the four introductions of *T. subterraneum* germplasm from Sardinia that were directly released as new cultivars in Australia in the early 1990's, *viz.* cultivars Denmark, Goulburn, Leura, and York. Against important diseases on *T. subterraneum* in Australia, two of these cultivars had good resistance to both the old and the new races of *Kabatiella caulivora*, two had good resistance to *Uromyces trifolii-repentis*, three had resistance to *Cercospora zebrina* and all four had outstanding resistance to the original race of *Phytophthora clandestina* (MJ Barbetti, unpubl.). This is despite the fact that these diseases occurred infrequently (e.g., *C. zebrina* and *U. trifolii-repentis*) or have never occurred (e.g., *K. caulivora* and *P. clandestina*) in Sardinia, highlighting the value of seeking out further sources of resistance to necrotrophic soil-borne pathogens from the Mediterranean centre of origin, even if the particular diseases of interest do not occur there (Barbetti, 1996, Nichols *et al.*, 1996, M.J. Barbetti, unpubl.). It is essential that germplasm from these Mediterranean regions be targeted in the search

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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for new annual and perennial pasture legumes and grasses with improved host resistance to both soil-borne fungal and nematode diseases.

### 8.2 Pasture Grasses

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Clarke and Eagling (1994) reported that '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.' It is clear that this perception currently continues, despite some evidence that exists to show the detrimental effects of soil-borne pathogens in pasture grasses. Unfortunately, there is now little pasture grass pathology skills available in Australia, to the extent that Australia may not be able to resource an adequate response to dealing with current soil-borne diseases currently faced or to any possible introduction of exotic pathogens of grasses.

### 8.3 Nutrition Soil-borne Pathogen Interactions

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

## 9 Recommendations for Future Research

### 9.1 Diseases of New Legumes

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While some work has already been initiated with *Rhizoctonia* attacking new pasture legumes in Western Australia (M.P. You, unpubl.), we need to know for across southern Australia how differential crops and different pasture legumes respond to field strains of each of the most important soil-borne pathogens. Specifically, we firstly, need to know how these strains of key pathogens (e.g., *Rhizoctonia*) on other rotational crops affect new pasture legumes and, secondly, how highly

## **Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems**

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susceptible new legumes can affect the inoculum levels of pathogens that are also virulent on rotational crops (e.g., Faba Bean, lupin, chickpea, pea, etc) that come after them.

### **9.2 Nutrients and IPM**

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The possibility of improving IPM of soil-borne diseases caused by necrotrophic pathogens by nutrient amelioration is an area that offers significant potential. Specifically, the critical role of nutrients and their effects on the severity of disease, the expression of host resistance, the interaction between host and pathogen and/or antagonists, as well as the critical role of nutrients on the inoculum carry-over are areas that offer significant potential. At best, currently such areas are under-evaluated not only as a means of enhancing host growth and defences but also for the provision of appropriate nutrient levels that could maximise the effective buffering activity of the biological antagonists of pathogens in soils across southern Australia.

### **9.3 Pathogen Complexes**

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It has been established that pathogen complexes bring about a synergistic effect on the extent of the diseases they cause on legumes. Diseases of pasture species caused by such complexes warrant special management strategies as the responses of individual pathogens to fungicides and cultural practices differ. Pathogen complexes in subterranean clover have been adequately dealt with but, in relation to soil-borne diseases of annual medics, grasses and new pasture legume species in particular, there is a need to characterise and understand the pathogen complexes occurring such that appropriate control measures can be implemented.

### **9.4 Loss Assessments**

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While there has been considerable research that highlights the impacts of soil-borne pathogens of subterranean clover, the relative impacts occurring across southern Australia for major soil-borne pathogens of annual medic, lucerne, new legumes, and grasses need to be defined such that future research investment can be directed and targeted at those pathogens causing the greatest impact on pasture productivity. Little or no information exists on the losses incurred by native and introduced grasses in pastures in southern Australia.

### **9.5 Mycotoxins and Quality**

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Mycotoxins produced by necrotrophic soil-borne fungal pathogens (e.g., *F. acuminatum* and *F. avenaceum*, associated with annual medic, subterranean clover and grass pastures in Western Australia) pose a potential significant threat to both to feed quality across all pasture legume and grass species because of their effects on animal productivity and to human food quality. The full extent of these threats need to be urgently assessed.

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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### 9.6 Host Resistance in Annual Medics and Grasses

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Because chemical control is expensive and cultural control is not feasible, the best option for disease control in new legumes, annual medics, lucerne and in annual and perennial grasses is to locate and utilise host resistance, as has been successfully achieved for subterranean clover in the past. However, a sound understanding of host resistance responses in terms of sub-specific pathogen variability (e.g., races of *Phytophthora clandestina*) is critical if the role and full potential host resistance offers within IPM is to be maximised. Current interest in studying the genetics of disease resistances in *M. truncatula* is expected to help in relation to a wide variety of diseases affecting a spectrum of species of pasture legumes.

### 9.7 Training

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There is an urgent need to train personnel for pasture pathology relevant to southern Australia. The experienced personnel of the past few decades (e.g., Stovold, Murray, Clarke, Nicholas, Chatel Flett, Gillespie, Greenhalgh, Taylor, Pankhurst, Francis, Barbetti) have already or are soon to retire or have transferred to other responsibilities.

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## **11 Summary Report for Researchers, Advisors and Producers**

### **KEY POINTS TO ADDRESS:**

- Identify all major pathogens, including fungi, oomycetes and nematodes
- Identify soil conditions that favour individual pathogens and their complexes
- Identify the role of the different host species in rotation to determine their susceptibilities and carryover potentials
- As no single management practice will control all root diseases, identify the impact of each management practice on each pathogen and their complexes
- Establish integrated pest management strategies, including use of fungicides, to manage the individual pathogens and their complexes

### **THE IMPACT OF PASTURE ROOT DISEASES ON PASTURE ESTABLISHMENT, PRODUCTION, PERSISTENCE INCLUDING THE BEST CONTROL OR MANAGEMENT PRACTICES CURRENTLY AVAILABLE**

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

#### **11.1 Soil-borne Disease Impacts**

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Root rot has seriously adversely affected the production from subterranean clover pastures over the past 15-20 years.

In Victoria, the problem was first recognized in 1960 and is a serious problem which reduces the productivity of pastures. A survey of subterranean clover pastures in 1970 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. A subsequent study published in 1994, showed that decline in permanent pastures

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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in north-eastern Victoria remains an on-going problem. Drenches of fungicides metalaxyl (against *Pythium* and *Phytophthora*) and benomyl (against *Fusarium*) in the 1980's demonstrated increases of nearly 60% in May and more than 95% in October in herbage production were obtained from where the fungicide application were made. In 1990, multiple treatments including P, Mo, lime pelleted seed, lime additions, metalaxyl (against *Pythium* and *Phytophthora*), lime+Mg+trace elements showed increases in herbage yields of the order of 10-12% from these treatments.

In N.S.W., the 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. Investigations in the 1970's showed that root rots were an important factor in this observed decline of established subterranean clover pastures. In NSW, in the 1980's, applications of the fungicide Ridomil used to control *Pythium* and *Phytophthora* in subterranean clover pastures determined increases in herbage yield of up to 58% as a consequence of reductions of root disease damage of up to 82% from this fungicide application.

In Western Australia decline has been reported since first recognised in the 1960's, with large areas in the south-west and south coastal districts have been affected by pasture decline due to root rot, 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. More than 90% of seedlings can be killed pre-emergence. Even on the surviving plants, reductions in plant size from root rot exceeded 70% suggesting that production from pastures with severe tap root rot may be very poor.

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

### 11.2 Important Soil-borne Pathogens

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A number of nematode and necrotrophic soil-borne pathogens have been associated with significant productivity decline and pose a serious threat to one or more annual or perennial forage legume or grass species to the extent that they require reseeding. For example, for fungal diseases subterranean clover and/or on annual medics, *Phytophthora clandestina*, various *Pythium* species such as *P. irregulare* in particular but also *P. ultimum* and *P. spinosum*, *Aphanomyces eutichies*, *Rhizoctonia solani*, and one or more of various *Fusarium* species such as *F. avenaceum* in particular but also *F. acuminatum*, are of concern. Other important soil-borne necrotrophic pathogens on annual pasture legumes may also include pathogens such as *Phoma medicaginis* and *Cylindrocarpon didymium* in specific locations.

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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

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

### 11.3 Disease Management - Options

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

#### *Host Resistance*

The utilisation of resistant cultivars offers the most cost-effective, long-term control measure for pasture diseases, 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.

Subterranean clover: The most promising avenue for disease control so far has been the development and use of cultivars with increased field resistance to root rot. Identifying sources of resistance to individual root pathogens should allow the development of cultivars with further enhanced root rot resistance. Extensive field testing of subterranean clover cultivars for root rot resistance in root rot-affected areas of Western Australia has been conducted and a wide range of resistance has been observed. Cultivars with good field resistance to root rot are Daliak, Dinninup, Esperance, Junee, and Karridale. Larisa is known to have a moderate degree of field root rot resistance while Trikkala has less but still useful resistance. Cultivars Denmark, York and Goulburn, all released in the 1990's, all have resistance to the most commonly occurring race of *P. clandestina*. Cultivar Gosse released in the 1990's, Riverina, along with the more recently released

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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cultivars of Napier and Coolamon, all have good resistance to more than one of the most common races of *P. clandestina*. Unfortunately, the recently released cultivar Urana is quite susceptible to the two most commonly occurring races of *P. clandestina*.

Ideally, identification of resistance to more than one pathogen, and/or to more than one pathogen race, in a single genotype or cultivar is ideal. Recent work in Western Australia has shown that for 12 genotypes of subterranean clover, resistances to clover scorch disease were related to those shown to major root pathogens, viz. one or more of *Phytophthora clandestina*, *Pythium irregulare*, and *Fusarium 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 cultivars with effective resistance to a range of pathogens that commonly occur in southern Australian annual legume pastures. Identification of pasture legumes with multiple resistances may be particularly critical in the future, especially as recent studies in Western Australia have shown that there are a total of eleven races of *Phytophthora clandestina* and presented the first clear picture of the racial distribution of *P. clandestina* in Western Australia that will be important both to breeders and to farmers. Differences were found in the distribution of *Phytophthora clandestina* race populations between Australian states will provide a sound basis not only for the selection/breeding of appropriate cultivars for specific regions of Australia to counter the predominant race populations, but also as a basis for enforcing quarantine measures in relation to seed movements within and outside Australia.

Annual medics, lucerne and grasses: The same opportunities as with subterranean clover also exist for at least some diseases, such as Phoma blackstem disease, in annual medics and lucerne, providing large scale screening of germplasm for resistance is undertaken as has been done for many years against clover scorch in Australia.

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. In Australia, useful resistance to post-emergence damping-off caused by *Pythium* spp. has been identified in *M. denticulata*. While only low levels of resistance to *P. clandestina* has been reported to date in Australia, such as for *M. rugosa* and *M. scutellata*, the existence of high levels of resistance to one or more races of this pathogen in subterranean clover 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. This may be related to the proportionately smaller acreages sown to annual medics compared with subterranean clover. 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 on annual medics as remains the case with subterranean clover. Realistically, there is a need for commercial cultivars 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.

It is clear that all new annual medic and lucerne cultivars released for regions where disease is common, such as across southern Australia, should possess adequate resistance to the major pathogens. In Australia, despite the identification of a number of sources of host resistance, breeders are generally yet to deliver this benefit to growers by way of more disease resistant cultivars.

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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Almost nothing is known in relation to potential host resistances to soil-borne pathogens that is available in grasses in Australia.

### *Cultural control strategies*

Grazing: While the normal grazing of subterranean clover, annual medic, and lucerne pastures has probably provided substantial disease control of foliar diseases it probably offers much less benefit towards control of soil-borne diseases. In some situations, however, grazing may damage plants and make them more susceptible to some soil- and trash-borne diseases, such as occurs with *Phoma* blackstem disease on annual medic and lucerne and with some crown rots of lucern in Australia and as likely occurs for some root disorders on subterranean clover (e.g., *Phytophthora clandestina* root rot).

Tillage and burning: Tillage can successfully reduce the impact of soil-borne diseases in pasture legumes, as demonstrated for subterranean clover. Field experiments in the 1970s in south western 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. 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. As many of the root pathogens on subterranean clover, lucerne and annual medics are common across these genera, it is likely that similar responses to tillage could be expected for annual medics and lucerne. In Victoria, it has been shown that simulated cultivation of soil in cores could also significantly reduce root rots in the dryland pasture soil but only if they had little surface litter.

Nutrition: Nutrition is an important component of disease management, with all fundamental mineral elements reportedly influencing disease incidence or severity. It is frequently the case that plants with good nutritional status are more likely to be better able to resist and/or tolerate diseases. 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 to severity. Fertiliser application rates to annual 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. However, studies in Western Australia suggested that the interaction of nutrition and soil-borne pathogens in pasture legumes may be quite complex.

Rotations: Pasture legumes form an integral component of cropping rotations because they give a high yield of good quality forage and are tolerant of grazing, 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, while still providing a feed base for animal production. Rotations not only provide 'disease break' benefits for the pasture legume phase itself but more importantly also for the rotational crop species, such as the reduced level of disease caused by *Rhizoctonia* in wheat in southern Australia. In two field trials in Western Australia, complete removal of subterranean clover for one season or, in particular two seasons, significantly reduced root disease in the immediate following year in which subterranean clover was allowed to regenerate. However, this effect also

## Role and Impact of Diseases Caused by Soil-borne Plant Pathogens in Reducing Productivity in Southern Australian Pasture Systems

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persisted poorly beyond the first season of regeneration and the losses in terms of subterranean clover herbage and seed yield during the period of subterranean clover removal may not be offset by subsequent benefits from root disease reductions. Despite this, 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 southern Australia, providing a suitable alternative pasture species can be cultivated and grown during the non-clover phase.

### *Fungicidal control strategies*

Fungicides are unlikely to provide effective or economic control of soil-borne diseases of pastures. In Victoria, it was shown that metalaxyl could provide useful control of root disease, especially that caused by *P. clandestina*, in the same way as was for applications of potassium phosphonate, primarily against this same pathogen. Similar results have been demonstrated for Western Australia. However, disease control with fungicides will become even less important if cultivars with improved resistance are deployed, but effective fungicides need to be available, along with appropriate cultural methods, if such resistance is overcome by changes in the pathogen populations.

While fungicide treatments and manipulation of management practices are not effective enough to make their use economically justifiable for very susceptible cultivars, it is possible that they may have a place in an integrated control system incorporating cultivars with at least some resistance to root rot.

### *Integrated disease management*

Integration of different control strategies should substantially improve the degree of control for both parasitic nematode and soil-borne fungal pathogens of subterranean clover, medics, lucerne, grasses. Integration of control strategies is already a focus of managing some of the most important diseases of subterranean clover, will remain a key focus for control of pasture diseases in new legumes, medics, lucerne and grasses, until new pasture cultivars with improved host resistance to parasitic nematodes and fungal pathogens are available, and, even then, will remain important should such host resistance succumb to more virulent races of one or more these pathogens that affect pasture species.