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REVIEW

Management of root diseases of annual pasture legumes in Mediterranean ecosystems - a case study of subterranean clover root diseases in the south-west of Western Australia

Martin BARBETTI[1,3], MingPei YOU[2,3], Hua LI[2], Xuanli MA[1], and Krishnapillai SIVASITHAMPARAM[2]

[1] School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, Nedlands WA 6009 Australia
[2] School of Earth and Geographical Sciences, Faculty of Natural and Agricultural Sciences, The University of Western Australia, Nedlands WA 6009 Australia
[3] Department of Agriculture and Food Western Australia, Baron-Hay Court, South Perth, WA 6151, Australia

Summary. Subterranean clover (*Trifolium subterraneum*) is an important component of Mediterranean dryland pasture ecosystems, such as in the south-west of Western Australia, where it is utilised as a winter annual pasture that provides nitrogen as well as disease breaks for rotational crops. Necrotrophic soil-borne fungal pathogens dominate Mediterranean ecosystems because of the ease of survival of these pathogens on infested residues over the dry summer period, and because of low levels of microbial competition in the impoverished and nutrient-deficient soils characteristic of these regions that predisposes plants to root diseases. In addition to herbage and seed yield losses from soil-borne fungal and nematode pathogens, changes in botanical composition, in the number of regenerating plants, their persistence, and factors affecting feed quality are significantly affected. Further, where the causal organisms of the diseases on subterranean clover are also common on other rotational crops, the impact of these soil-borne pathogens appears far wider in Mediterranean ecosystems than previously considered. Under these conditions, soil-borne pathogens pose a serious threat to the productivity of this self-seeding pasture legume, to the extent that reseeding may become necessary. Pathogens such as *Phytophthora clandestina*, various *Pythium* species particularly *Pythium irregulare*, *Aphanomyces* sp., *Rhizoctonia solani*, one or more *Fusarium* species, *Phoma medicaginis* and *Cylindrocarpon didymium* are of concern, as are the nematode parasites from the genera *Meloidogyne*, *Heterodera, Pratylenchus, Trichodorus* and *Radopholus*. In this ecosystem, root pathogens operate together as disease complexes and the challenge therefore has been to source host genotypes with resistance to multiple pathogens. In addition to plant nutrition, environmental factors, in particular rainfall (soil moisture) and soil temperature, have a marked effect on both the disease severity caused by individual pathogens and on the interactions that occur between the different root pathogens. Approaches to disease control in this region include a range of management strategies. Cultural control strategies, including manipulation of grazing and rotations, offer some benefits. Manipulation of soil fertility also offers scope as this can enhance root physiology related to host resistance, overall plant growth and vigour, and also to improve the effective biological buffering against the pathogens. Fungicide treatments and manipulation of management practices may have a place in an integrated control system incorporating cultivars with useful resistance to root diseases. Clearly, host resistance offers the most cost-effective, long-term control, especially as resistance to several of these soil-borne pathogens has been identified. The Mediterranean Basin, which is the centre of origin of this pasture legume, has proved to be a productive source of resistance to soil-borne necrotrophic pathogens and is likely to be a source of new subterranean clover cultivars.

Key words: necrotrophic pathogens, management, fungi, nematodes.

Corresponding author: M.J. Barbetti
Fax: +61 8 64887077
E-mail: mbarbett@cyllene.uwa.edu.au

Introduction

Necrotrophic pathogens dominate regions with a winter-dominant rainfall, including those with a Mediterranean-type climate, because of the ease of survival of these soil and trash-borne pathogens on infested residues through the dry summer periods. The impoverished and nutrient-deficient soils across many parts of these regions predispose the plant host to these pathogens as there is often little microbial competition with the necrotrophic pathogens (Sivasithamparam, 1993). Hence, it is not unexpected that necrotrophic fungal pathogens provide significant challenges to productive utilisation of subterranean clover 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 of Australia with Mediterranean-type climates, such as south-west Western Australia and significant areas of South Australia and Victoria (Chakraborty *et al.*, 1998). Any increase in summer rainfall that occurs in conjunction with global warming will lead to increased rates of breakdown on infested residues and a more rapid decline in inoculum levels of necrotrophic pathogens. Since the widespread adoption of minimum tillage practices across south-west Western 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, which increase 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 oilseed rape across southern Australia.

Over 400 fungal, bacterial, viral, mycoplasma and nematode diseases are known to affect the productivity of pastures (Haggar *et al.*, 1984) and are known to play a role in affecting productivity of predominately annual pastures in Australia (Murray and Davis, 1996). The literature on the productivity of pasture legume species such as subterranean clover has been previously reviewed in relation to root disease research undertaken up to the mid 1980s (Barbetti *et al.*, 1986). Plant pathogens result in losses in subterranean clover that can adversely affect animal feed availability. Such losses affect industries such as wool, meat, dairy or grain production, and these losses have been reviewed to at least some extent previously in Australia (*e.g.*, Johnstone and Barbetti, 1987; Barbetti *et al.*, 1996). It is noteworthy that before 1970, diseases of pastures in Australia were not considered to be a significant problem and therefore were not thought to warrant control measures (Sloane *et al.*, 1988).

This review i), discusses the key characteristics of the agricultural ecosystems of Mediterranean regions and the relevance of pastures therein; ii), defines the role of subterranean clover in Mediterranean ecosystems in south-west Western Australia and the challenge posed to it from diseases; iii) addresses the impact of soil-borne fungal and nematode pathogens in these ecosystems in Western Australia and the difficulties in assessing their true impact; iv) describes the significance of the disease symptoms and the major fungal pathogens involved; v) describes the environmental influences on biotic stress imposed by fungal root diseases; vi) outlines the key soil-borne plant parasitic nematodes and their response to environmental conditions; vii) discusses the interaction of plant nutrition and soil-borne pathogens; viii) addresses the implications of diseases of Mediterranean ecosystems for rotational pasture and cropping systems; and, ix) addresses the approaches on the disease management made to date using chemicals, cultural practices, and host resistance. For this review, we have based our definition of the term "necrotroph" on that of Agrios (2004), to mean an organism that has at least one part of its life cycle on dead host/tissue and that can grow on artificial nutrient media.

Key relevant characteristics of the agricultural ecosystems of Mediterranean regions and the relevance of pastures there-in

The Mediterranean climate can best be described as "a transitional-regime temperate and dry tropical climate characterised by a concentration of rainfall in winter, occurrence of a distinct summer drought of variable length, high variability in precipitation from year to year, mild to warm or hot summers, and cool to cold winters with an absence of continental thermic excursion, and intensive solar radiation especially in summer" (Sivasithamparam, 1993). Even in spring, high rates of evaporation and high winds can lead to large deficits for agricultural activities (Cooper *et al.*, 1987).

While the Mediterranean regions are predominantly characterised by brown earths, brown and red Mediterranean soils and cinnamon soils (Bridges, 1970), variations in soil formation processes in these regions have resulted in a mosaic of soil types. It is mainly on the basis of different contents of plant nutrients in the soil that two groups of Mediterranean lands have been differentiated, *viz*., the moderately fertile soils of Chile, California and the Mediterranean Basin, and the infertile soils of South Africa and Australia. In these latter two countries, soils have developed from nutrient-poor, very old parent materials or infertile siliceous sands, have been highly weathered, and highly leached, and their levels of macro- and micro-nutrients have been greatly reduced (Sivasithamparam, 1993).

Annual pasture species, including subterranean clover, play an important agronomic role in dryland farming in Mediterranean-type regions where they are often an integral component of rotational grazing systems and/or cropping systems. They are particularly important in these regions, including the Mediterranean ecosystems of south-west Western Australia, much of South Australia and parts of Victoria, which not only have a typical Mediterranean-type climate, but where pasture legumes grow as winter annuals for animal feed that also provide both nitrogen and disease breaks for rotational crops, especially cereals and oilseed rape. Ley farming has been the traditional crop rotation system in Western Australia (Underwood and Gladstones, 1979), whereby the pasture phase is reliant on self-regeneration of annual legumes from hardseeds which remain dormant during a cropping phase of 1 or 2 years. Since the 1970s grain legumes, particularly lupins, field peas and faba beans, have been increasingly incorporated into many of these cropping rotations in place of pasture legumes (Nichols *et al*., 2007). In higher rainfall and irrigated areas, pastures tend to be permanent or semi-permanent, supporting meat, wool and dairy production (Nichols *et al*., 2007).

Role of subterranean clover in the Mediterranean ecosystems of south-west Western Australia and the challenge from diseases

Rainfed subterranean clover is the most important pasture legume in the Mediterranean regions of southern and eastern Australia (Morley, 1961; Powell, 1970; Gladstones and Collins, 1983) particularly between latitudes 30 to 39°S and were sown over an estimated area of 16 million ha or more of dryland pasture (Gladstones, 1975; Cocks *et al*., 1978; Collins and Gladstones, 1984) by the mid-1970's. The area sown has more recently been revised upwards to over 22 million ha in Australia (Sandral *et al*., 1997). 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). Even in the Mediterranean ecosystems of south-west Western Australia, by the mid 1970's some 6 million ha of subterranean clover were sown (Gladstones, 1975).

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 management practices, 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 water-logging (Gladstones, 1975).

In recent years, there has been a growing emphasis on improving the productivity of annual subterranean clover-based pastures by intensifying grazing and feed-base utilisation systems (Barbetti *et al*., 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 the individual cultivar potentials. This is particularly noticeable in areas where the utilisation of monocultures or near-monocultures in short leys is increasing, for while such cultures often increase yield, they do not provide the buffering benefits that result from having other genera present, and consequently losses from diseases are exacerbated.

Losses in subterranean clover from foliar disease (Barbetti, 1987; Barbetti and Nichols, 1991; Barbetti, 1996) have generally been better quantified than those for soil-borne diseases. Direct losses from disease in subterranean clover can 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.*, 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 the increased cost and side-effects of control (Barbetti *et al.*, 1996). In particular, mycotoxins produced by necrotrophic soil-borne fungal pathogens (*e.g.*, *Phoma medicaginis* [Mortimer *et al.*, 1977; M.J. Barbetti, unpublished data], *F. acuminatum* and *F. avenaceum* [Barbetti and Allen, 2005, 2007]), associated with legume pastures in south-west Western Australia pose a significant threat to feed quality in this Mediterranean ecosystem because of their effects on animal productivity and, as a consequence, potentially to human food quality. The full extent of these threats from mycotoxins has yet to be assessed.

Pasture legumes such as subterranean clover contribute to the saprophytic survival of fungal pathogens, including those of rotational crops, by providing the soil nitrogen necessary for saprophytic growth and colonisation. In particular, soils with low C:N ratios tend to favour saprophytic survival of several soil-borne cereal and legume pathogens (Garrett, 1970).

It has been established that pathogen complexes have a synergistic effect on the severity 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.

One of the major challenges is to manage such disease complexes under the traditional rotation systems utilised under Mediterranean ecosystems where there can be both long pasture legume phases and/or rotation with different crop species that are also hosts to these same pathogens on pasture legumes. This aspect is addressed further in a specific section of this review.

Impact of soil-borne fungal and nematode pathogens in Mediterranean ecosystems in Western Australia and the difficulties involved in assessing the nature of the impact

Root rot has seriously affected production from subterranean clover pastures in Mediterranean ecosystems in south-west Western Australia for several decades (Johnstone and Barbetti, 1986) and some of the impacts have been defined in earlier reviews (*e.g.*, Barbetti *et al.*, 1986).

In south-west Western Australia, pasture decline was first recognised by Shipton (1967). Large areas were found to be 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 large percentage of seedlings were killed even prior to emergence, and where emerged seedlings died from root rot in the first few weeks of the growing season (Barbetti and MacNish, 1983). Wong *et al.* (1985b) reported 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 to 7 weeks after emergence till 16 to 17 weeks into the growing season. Such reductions often exceeded 70%, suggesting that yield from pastures with severe tap root rot may be very poor. Barbetti (1984e) 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.

While there is a considerable amount of information on soil-borne pathogen-induced losses in subterranean clover, a significant proportion of this only comes from experiments involving glasshouse or controlled environment conditions (*e.g.*, Barbetti and Sivasithamparam, 1987). While showing the capacity of a pathogen to cause damage, such studies are done under conditions that are not directly related to what happens in grazed commercial subterranean clover pastures and the information provided cannot be reliably extrapolated to such pastures. There is a need to assess soil-borne fungal and nematode-induced losses in field situations that allow the data obtained to be used to make rational disease management decisions and economic assessments. Grazed monoculture first year swards could provide such information, providing more relevant data than those obtained under glasshouse or controlled environment conditions. Relatively little work has been done to date in regenerated commercial subterranean pastures. This is probably because their use involves more complex assessments, mainly due to the presence of more than one plant species in these pastures.

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 in subterranean clover occurring across south-west Western 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 a limiting factor. 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, persistence after the first year, rotational effects (such as nitrogen availability) and factors affecting feed quality (such as production of phyto-oestrogens and mycotoxins). There would be significant benefit from improvement of economic evaluation packages or models, from making them more user-friendly, and in particular from employing them more widely in relation to pasture diseases (Barbetti *et al.*, 1996). There should be wider appreciation of the need for better management of soil-borne pathogens and the benefits to subterranean clover pastures that could be derived from such management.

Significance of disease symptoms and fungal pathogens involved

Root rots of subterranean clover are widespread in the Mediterranean ecosystems of south-west Western Australia. The above-ground symptoms of root rots in subterranean clover vary in different locations across this region, most likely because of peculiar environmental and/or nutritional differences (Barbetti and MacNish, 1983). Root disease on subterranean clover disease can be evident as stunted yellow-green, yellow-red, or red-purple plants (Barbetti, 1983c; Barbetti and MacNish, 1983) scattered among apparently healthy plants, or the affected areas may occur in distinct patches (Barbetti and MacNish, 1983). In some situations, restricted areas of subterranean clover within a paddock may be diseased (Barbetti and MacNish, 1983), while in others whole paddocks may be affected (Barbetti and MacNish, 1983). The internal and external tissues of diseased roots are usually brown and discoloured. In south-west Western Australia, Barbetti and MacNish (1983) noted root symptoms involving part or all of the root system with the tap root usually being more severely damaged than the laterals. This is possibly because the tap root is the first root system to be established on subterranean clover seedlings and it has to penetrate the upper soil layers that contain most of the pathogen inoculum in these ecosystems. This perhaps also explains why MacNish *et al.* (1976) reported a rot of the tap root confined to 10 to 20 mm below the crown in subterranean clover. Sometimes affected plants produce new lateral roots above the lesions on the tap root, and these plants may slowly recover (Barbetti and MacNish, 1983) if there are sufficient rainfall events for this to occur. Plants with severely damaged root systems frequently show stress symptoms (*e.g.*, yellow or red colour of foliage, wilting), and these symptoms are frequently exacerbated by the infrequent rainfall associated with Mediterranean ecosystems.

A number of soil-borne fungi have been shown to have varying degrees of direct involvement in causing root disease of subterranean clover in the Mediterranean ecosystems of south-west Western Australia. These include *C. didymum* (Barbetti, 2005), *F. oxysporum* (Shipton, 1967; Barbetti and MacNish, 1978; Wong *et al.*,1984; Wong *et al.*,1985b; Wong *et al.*, 1986f), *F. avenaceum* (Shipton, 1967; Wong *et al.*,1984; Wong *et al.*,1985b), *F. graminearum* (Shipton, 1967), *F. moniliforme* (Shipton, 1967), *F. culmorum* (Wong *et al.*,1985b), *F. equiseti* (Wong *et al.*,1985b), *M. phaseoli* (Wong *et al.*,1985b), *Phoma medicaginis* (Wong *et al.*,1984; Wong *et al.*,1985b), *Pythium irregulare* (Barbetti and MacNish, 1978; Wong *et al.*,1984; Wong *et al.*,1985b), *P. debaryanum* (Barbetti and MacNish, 1978), *P. acanthicum* (Barbetti and MacNish, 1978), *P. middletonii* (Barbetti and MacNish, 1978), *P. spinosum* (Wong *et al.*,1985b) *Phytophthora clandestina* (Taylor *et al.*, 1985a; Wong *et al.*, 1985b, Wong *et al.*, 1986b,c), *Rhizoctonia* spp. (Barbetti and MacNish, 1978; Wong and Sivasithamparam, 1985; Wong *et al.*, 1985b), in particular *R. solani* (Wong *et al.*, 1984; Wong and Sivasithamparam, 1985; Wong *et al.*, 1985b) but also *R. cerealis* (Wong and Sivasithamparam, 1985), and *Waitea* spp. (Wong and Sivasithamparam, 1985; Wong *et al.*, 1985b). You *et al.* (2005d) characterised a total of ten races of *P. clandestina*. Also in south-west Western Australia, fungi such as *F.*

avenaceum, *Leptosphaerulina trifolii*, *Myrothecium verrucaria*, and *P. medicaginis*, commonly isolated from subterranean clover foliage, are all reported to cause some root disease under non-competitive conditions (Barbetti, 1984c), in environments commonly associated with the impoverished soils of this region.

In south-west Western Australia, Barbetti and MacNish (1978) showed that three frequently isolated fungi; *viz*. *P. irregulare* in particular, *P. acanthicum*, and *F. oxysporum* caused the most severe root rot and most strongly reduced seedling emergence, particularly following inoculation with two or more fungi in combination rather than a single fungus. Similarly, Wong *et al*. (1984) showed that various combinations of *F. avenaceum*, *P. irregulare*, *R. solani*, *F. oxysporum* and *P. medicaginis*, increased the severity of root disease and decreased plant survival and plant weight more than when these pathogens were tested singly. Wong *et al*. (1984) also showed that *P. clandestina* interacted with *F. oxysporum* to produce more severe root rot than did either fungus alone. Root rot of clover clearly involves a complex of fungi which interact not only with each other but also with the biotic and abiotic environment surrounding them. A range of different fungi is normally associated with diseased subterranean clover roots and no one fungus has been able to reproduce the whole range of different field disease symptoms observed in different locations. Investigations which implicate a single fungus as the cause of a disorder have often been conducted solely with that particular fungus. That a number of different fungi are present on diseased roots, and that they interact to enhance disease has been 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* sp., *Rhizoctonia species* and *F. avenaceum*. However, it is likely that additional fungi and fungal complexes will be found associated with diseased roots in the future as new isolation procedures are applied. Pathogens such as *P. clandestina* and *Aphanomyces* sp. are rarely isolated in routine isolations even from sites known to harbour these pathogens. Molecular probes are expected to be more reliable indicators of the activity of these pathogens in clover roots.

Enhanced pathogenesis due to phytotoxins and/or mycotoxins in the 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 disease, as could mycotoxins produced by individual pathogens. For example, where *C. didymum* is found in south-west Western Australia, the frequently observed stunted appearance of the tap and lateral roots of *C. didymum*-affected subterranean clover could possibly be a consequence of the highly toxic mycotoxin brefeldin A produced in the root tissue by this pathogen (Barbetti, 2005).

Certain fungi associated with root rots of subterranean clover can be seed-borne, including *Fusarium* spp. and *R. solani* (MacNish, 1977). Seed-borne infestation is important for biotrophs but less important for necrotrophs where seed-borne infections are important only in relation to the introduction of new pathogens or their races to new areas. This is largely because necrotrophs in Mediterranean ecosystems readily establish during the pasture and/or cropping season, and their inocula are conserved over the dry summer period in the soil organic matter, and in some instances in infested plant residues above ground. The lack of any relationship between root rot incidence and fungi associated with seed is an indication that seed transmission is unimportant in the aetiology of root rots of subterranean clover in south-west Western Australia (MacNish, 1977). This view is supported by Barbetti (1984a) who showed that removal of fungi from subterranean clover seed had no influence on the severity of root diseases after sowing.

Environmental influences on biotic stress imposed by fungal root diseases

There have been a number of attempts to relate environmental factors to the severity of root diseases of subterranean clover ecosystems in south-west Western Australia. For example, analysis of climatic data for centres along the south coast from 1972 to 1975 showed that 1973, a particularly severe root disease year, had significantly heavier and more frequent rain after the break of season than did the other years when root disease severity was much lower (MacNish *et al*., 1976). This is not surprising, as key oomycete pathogens such as *P. clandestina*, *Aphanomyces* and various *Pythium* spp. are all strongly favoured by wet soil conditions.

Also in south-west Western Australia, Wong *et al*. (1984) investigated how soil moisture (45% water holding capacity [WHC], 65% WHC, and flooding) interacted in relation to the pathogenicity of *F. avenaceum*, *F. oxysporum*, *P. medicaginis*, *P. irregulare*, and *R. solani*, both alone and in combination. It is noteworthy that these fungi and their combinations caused root disease particularly over the range of soil moisture conditions that approximated to those occurring in root rot-affected fields in south-west Western Australia. The most severe root rotting occurred at 65% WHC, with less at 45% WHC, and least under flooding conditions. Conditions of 65% WHC would frequently be expected to occur in the higher rainfall zones of the south-west Western Australian Mediterranean ecosystem, favouring the oomycete pathogens. In contrast, drier soils favoured pathogens such as *Fusarium* spp. and *R. solani*. While significant rainfall events may trigger serious attack by oomycete pathogens, these same rainfall events are also needed for compensatory root growth following attack by one or more soil-borne pathogens. Wong *et al*. (1986e) showed that an osmotic water potential of -12 bars prevented mycelial growth and that optimal growth for *P. clandestina* was 0 bar. Hence it was not surprising that You *et al*. (2006) found that rainfall levels were important determinants of the race distribution for *P. clandestina*, with the majority and most diverse of *P. clandestina* races occurring in the high rainfall zone with 700–1000 mm annual rainfall. Such an association of races of this pathogen with a particular rainfall zone is important in relation to the selection/breeding and the deployment of subterranean clover cultivars for particular rainfall zones in south-west Western Australia. While other root pathogens such as *Pythium* spp. and *R. solani*, are also found across these same rainfall zones, *Pythium* spp. appear to do most damage in the higher rainfall zones while *Rhizoctonia* spp. do more damage in the medium to lower rainfall zones (M.J. Barbetti, unpublished data).

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. Wong *et al*. (1985b) showed that the growth rate of *P. clandestina* increased with the progressive increase of the incubation temperature up to 20°C. Wong *et al*. (1986e) showed that saprophytic survival of *P. clandestina* in pasteurised soil was greater under cooler conditions (5–10°C), and°C). Wong *et al*. (1986c) found that the most severe root disease occurred at a soil temperature of 10°C, followed by 15 and 20°C, coinciding with autumn/early winter conditions of the Mediterranean ecosystems of south-west Western Australia. More recently, Barbetti (2005) demonstrated that *C. 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 high rainfall coastal area of south-west Western Australia during the winter months when this pathogen appears to be most common.

There was a significant interaction between temperature and moisture for the various fungi and fungal combinations tested in Western Australia by Wong *et al*. (1984). These researchers looked at how soil moisture and soil temperature interacted in relation to the pathogenicity of *F. avenaceum*, *F. oxysporum*, *P. medicaginis*, *P. irregulare*, and *R. solani*, both alone and in combination. It is noteworthy that these fungi and their combinations caused root disease particularly with the combination of soil moisture and temperature conditions that approximated to those occurring in root rot-affected fields in south-west Western Australia during germination and early growth. Subsequently, Wong *et al*. (1986c) demonstrated that *P. clandestina* caused pre- and post-emergence damping-off in subterranean clover under a range of soil temperatures (10, 15, 20 and 30°C) and moisture conditions (65 and 100% WHC and flooding), with the greatest reductions in seedling survival occurring in saturated and flooded soil. Similarly, Wong *et al*. (1986b), showed that there was a marked reduction in the growth of *P. clandestina* with increasing water stress across the range of temperatures that could be expected in south-west Western Australia, suggesting that for pathogens such as *P. clandestina*, water stress is more important than the variations in temperature expected to occur in that region. Wong *et al*. (1986e) showed that while saprophytic survival of *P. clandestina* in pasteurised soil was optimal under cooler conditions (5–10°C) at all water potentials tested (-0.01, -0.026, and -0.063 bars), yet at higher temperatures (15–32°C) *P. clandestina* survived much better in wetter soil. Clearly there are different moisture-temperature interactions for different individual pathogens.

In a study by Barbetti (1990), changes in the soil pH (by the addition of lime) affected seedling survival and the levels of rot of the tap and lateral root systems by *F. avenaceum*, *P. clandestina*, *P. irregulare* and *R. solani*. The different root pathogens often responded differently to the addition of the lime, and the relative cultivar resistance-rankings to individual pathogens sometimes varied widely depending on the amount of lime added. There was a clear interaction between lime and cultivar and between lime and pathogen for all parameters measured and lime shows potential for management of root disease. Further evidence of the effect of pH was provided by Wong *et al*. (1986b) who investigated the effects of temperature, pH and water potential on the growth or survival of *P. clandestina* and found that while this pathogen grew on agar over a pH range of 4–9 its growth rate increased as the pH of the medium rose from 4 to 6 at temperatures of 15, 20, and 25°C.

Studies on the detection of *P. clandestina* on infected subterranean clover roots over time (Wong, 1986) showed that the pathogen was detected most readily two weeks after germination, its activity gradually declining after this phase. This is the phase when the host is most susceptible and the high incidence of the pathogen and the presence of susceptible host (*i.e*., the plant and pathogen cycles) coincide by the very nature of the Mediterranean environment.

Wong *et al*. (1986a) studied the nature and behaviour of *P. clandestina* in the soil and found that the pathogen could only be recovered from soil screenings containing small root fragments. Exposure of *P. clandestina* inoculum to increasing numbers of soil microbes reduced the severity of root disease of subterranean clover, an indication that *P. clandestina* is favoured by the relatively poor biological buffering capacity of soils in south-west Western Australia. Wong *et al*. (1986a) 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 than for July to December, and that the number of propagules increased after January, to peak in May, the month in which root rot is often most severe (Wong *et al.,* 1986b), coinciding with the opening seasonal rains.

There may well be effects of herbicide application upon the levels of root disease that occur in subterranean clover. For example, Barbetti (1984d), examining intact cores from Western Australian root rot-affected fields, found that prior application of a paraquat/diquat mixture in the form of commercial Spray-seed® led to slight reductions in root disease severity even though it had no effect on the incidence of various soil-borne pathogens.

As outlined above, environmental factors such as rainfall, soil moisture and soil temperature clearly have a marked effect on both root disease severity in subterranean clover caused by 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. Ideally, additional field studies should be undertaken, as has been done for annual *Medicago* spp. by You *et al*. (1999) who looked at how rainfall, cultural practices, soil and plant nutrients in the field affected root disease and parasitic nematode numbers in south-west Western Australia. In eastern Australia, Smiley *et al*. (1986), using intact field cores, showed that root disease 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. This is a further indication that the fluctuating soil temperature and moisture conditions so common in Mediterranean ecosystems have a significant impact upon the expression of root disease in subterranean clover.

Key soil-borne parasitic nematodes and their response to environmental conditions

Meloidogyne javanica, *M. hapla*, *M. incognita, Pratylenchus* sp. and *Radopholus* sp. are potential pathogens of subterranean clover in south-west Western Australia (Shipton, 1967). More recently, surveys of sites of subterranean clover root disease in south-west Western Australia have indicated the presence of the genera *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 *M. 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 most comprehensive work on root-knot nematodes and their association with fungal root rot of 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 that *M. arenaria* occurred widely there. 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 disease on tap roots and found that *M. arenaria* was particularly pathogenic on subterranean clover at high inoculum levels (> 16,000 eggs per pot).

Pung *et al*. (1992b) conducted pot experiments to examine the effect that *F. oxysporum* and *M. arenaria,* applied singly or in combination, had on the root health and growth of subterranean clover. They found that while *M. arenaria* had no effect on root necrosis in either pasteurised or autoclaved soil, it still adversely affected plant growth. Further, Pung *et al*. (1991b) showed that the timing of infection and the proximity of the root tips of the host root system to infection with *M. arenaria* and *F. oxysporum* appeared to be the major determining factors of root growth and disease development in plants exposed to these pathogens. For example, they found that the induction of galls by the nematode and early infection by *F. oxysporum* resulted in severe inhibition of root growth, particularly of the lateral roots.

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 by 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 developed and reproduced more rapidly while the soil temperature was still relatively high (> 15°C) in May–June, coinciding with the opening seasonal autumn–winter rainfall events. The second generation developed as the soil temperature increased between September and November and as rainfall events rapidly decreased. These findings were consistent with observations from the pot experiment, in which *M. arenaria* gall production and its development and reproduction in both the tap and lateral roots was 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 were important determinants of the severity of disease caused by *M. arenaria* in Mediterranean ecosystems. 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 and its preference for tap roots under more favourable conditions.

Interaction of plant nutrition and soil-borne diseases

Nutritional deficiencies such as those that occur in the old, leached soils of the Mediterranean ecosystems in south-west Western Australia are known to reduce the natural resistance of crop plants to disease (Graham, 1983). It is however generally accepted that diseases, especially those caused by soil-borne necrotrophs, are frequently exacerbated across south-west Western Australia by nutritional stress imposed on the plant hosts. While a correction of these deficiencies in most cases reduces the severity rather than limits the incidence of the diseases, it still offers some opportunities for exploitation. Plant nutrients can reduce the severity of diseases by enhancing plant growth and disease tolerance. Nutrients such as phosphates enhance host resistance by stimulating the production of phytoalexins (compounds produced by resistant plants challenged by the pathogen) (Gottstein and Kuc, 1989). The macro- and micro-nutrient status of the plant affects disease severity (Sadasivan, 1965; Graham, 1983) and this is an area that still remains to be explored for subterranean clover.

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 the host and the pathogen and/or the antagonist, each of which can also be affected independently by the availability of nutrients. Soil fertility can also affect disease development by its effect on the activities of the soil microflora that inhibit the soil-borne pathogens. Components of soil fertility have been associated with naturally occurring soil suppressiveness (Cook and Baker, 1983; Mukerji and Garg, 1988). For instance, a range of factors including organic matter, Ca, 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) and it is likely that the same applies to *P. clandestina* attacking subterranean clover. Soils in south-west Western Australia with relatively high levels of P, NO_3, or Fe were associated with an increased level of tap root disease in annual *Medicago* spp., while soils with a high pH were associated with reduced disease in the tap roots (You *et al.*, 1999). As illustrated in Fig. 1, soil nutrients can affect diseases by their effects on the nutrition of the plant host or on the microbial antagonist(s).

Pathogens such as *R. solani* are capable of extensive spread in the soil by utilising the available organic debris for energy (Garrett, 1963). This has been aptly termed 'combative migration' whereby colonization occurs in the presence of, and often in competition with, other soil micro-organisms. In the soils of south-west Western Australia, which are nutrient poor and low in microbial activity, even pathogens such as the 'take-all' fungus (*Gaeumannomyces graminis* var *tritici*), which has a low saprophytic ability (Garrett, 1963), can succeed in combative migration in a Mediterranean environment (Glenn *et al.*, 1988). This certainly indicates that the inoculum potential of certain soil-borne pathogens during the saprophytic colonization phase can be reduced by manipulating the nutrient and microbial status of soils supporting subterranean clover pastures in the Mediterranean ecosystems of south-west Western Australia.

The use of nutrient amelioration to improve the management of soil-borne diseases caused by necrotrophic pathogens is an area that has significant potential. Specifically, the critical role of nutrients and their effects on the severity of disease, the expression of host resistance, the interaction between

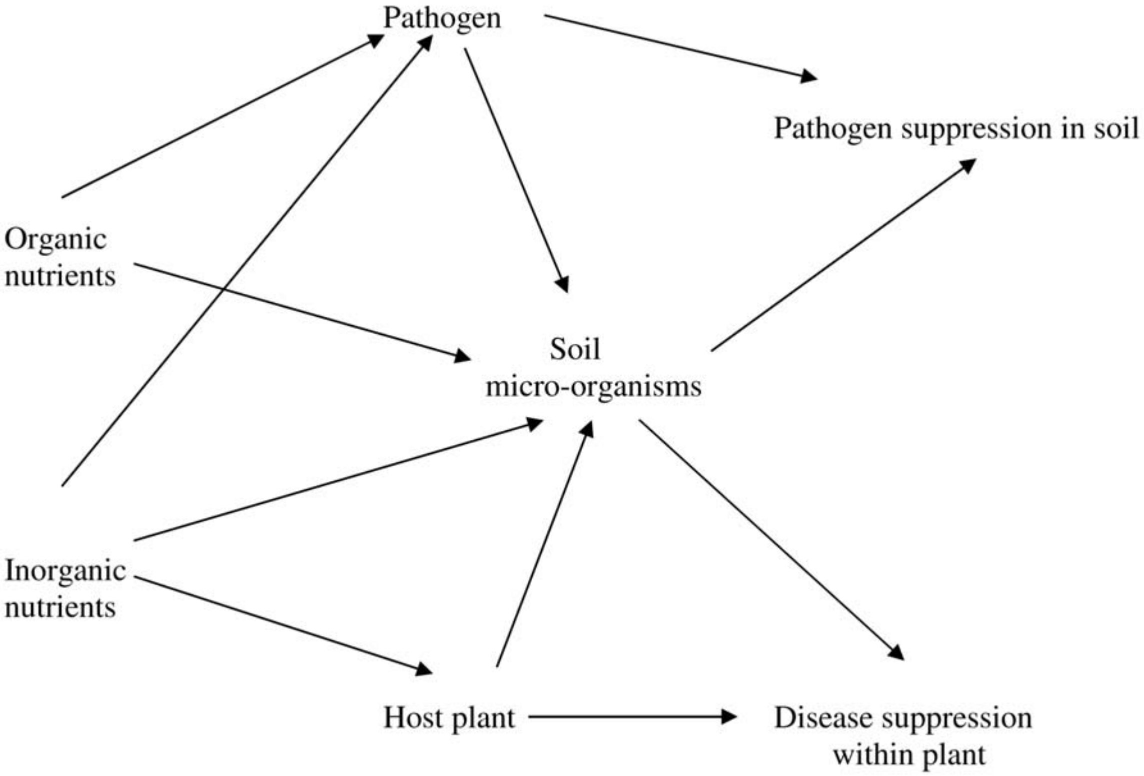

Fig. 1. Ways by which soil nutrients affect diseases by their effects on the pathogen, on the nutrition of the plant host or on the pathogen and/or microbial antagonist(s) resident in the soil. (Adapted from Sivasithamparam, 1996).

host and pathogen and/or antagonists, and the critical role of nutrients on the inoculum carry-over are areas that need to be investigated. Such areas are currently under-evaluated, to say the least, not only in terms of their potential to enhance host growth and defences, but also to provide appropriate nutrient levels that maximise the effective buffering activity of the biological antagonists of pathogens in the soils of the Mediterranean ecosystems of south-west Western Australia. It is evident that little is currently known about the interaction of nutrition with the major soil-borne pathogens of subterranean clover, and improved management of major soil-borne pathogens of pasture species across south-west Western Australia can be expected to occur from to a better understanding the full potential for manipulating nutrition for improved disease control.

Implications that diseases of pasture legumes in Mediterranean ecosystems have for rotational cropping systems

The role played by necrotrophic soil-borne fungal and nematode pathogens may in fact be far wider and have greater impact than previously considered. This is because there are many instances where diseases on subterranean clover are common to other rotational pasture species and/or crops. Examples of such shared pathogens include *Phoma medicaginis (*Khan and Barbetti, 1987) and *R. solani* (You *et al*., 2008). *P. medicaginis* can cross-infect between pasture legume and crop species (Barbetti and Khan, 1987; Sivasithamparam, 1993; M.P. You, unpublished data), perpetuating pathogen survival in the absence of a host during crop rotation cycles and reducing or even eliminating the expected disease-break benefit from rotating crop species. Similarly, some strains of *P. irregulare* can readily cross-infect between subterranean clover and one or more other pasture legumes and/or crop species including cereals (Sivasithamparam, 1993; M.P. You, unpublished data). In addition, there is a significant number of diseases that are common to subterranean clover and other widely cultivated pasture legumes such as annual *Medicago* spp. (Barbetti, 1989a,b; Tivoli *et al*., 2006); they include those diseases that are caused by *Pythium*, *Fusarium*, *Phoma*, *Rhizoctonia* and *Phytophthora* spp. In contrast, studies by Harvey *et al*. (2001) showed that host-mediated selection occurs in relation to cereal, annual *Medicago* spp. and subterranean clover isolates of *P. irregulare*, suggesting that there could actually be a limited potential in some situations to reduce the impact of such pathogens with a wide host range by the manipulation of rotational practices.

Studies have recently been undertaken on *R. solani* strains attacking subterranean clover and newly introduced pasture legumes in south-west Western Australia (M.P. You, unpublished data). However, there is a need to know how rotational crops and pasture species respond to field strains of each of the major soil-borne pathogens. Specifically, we need to know, first, how strains of key pathogens such as *R. solani* on other rotational crops affect subterranean clover and new pasture legume species and, second, how highly susceptible cultivars of subterranean clover and new legumes affect inoculum levels of soil-borne pathogens that are also virulent on rotational crops (faba bean, lupin, chickpea, pea, etc.) that are sown following the pasture phase of cropping.

Rotational crops also probably differ in the extent to which they influence the populations of specific rhizosphere organisms that compete, antagonise or suppress pathogens. For example, Cotterill and Sivasithamparam (1988) showed in the Mediterranean ecosystems of south-west Western Australia that the reduction in take-all in cereals with different crop rotations is not simply due to depriving the pathogen of its natural host, and that the biotic/abiotic factors associated with rotations may vary between crops. It is likely that a similar situation applies to subterranean clover pathogens. This situation may not apply to all pathogens. For example, Wong *et al*. (1986b) showed that subterranean clover residues were the sole source of *P. clandestina* inoculum in the pasture sward of mixed plant species not containing other pasture legume species. In this instance, while there were unlikely to be adverse implications for rotational crops except for annual *Medicago* spp. or *visa versa*, it was clear that the length of the disease break without the presence of subterranean clover may be important in determining the extent of inoculum carryover to the next subterranean clover pasture.

There are the side effects resulting from the production of mycotoxins as a consequence of infection by one or more necrotrophic soil-borne fungal pathogens such as *F. acuminatum*, *F. avenaceum*, and

F. graminearum associated with annual pasture legumes in south-west Western Australia (Barbetti and Allen, 2005, 2007). Such *Fusarium* spp. can readily cross-infect between and across different pasture (*e.g.,* annual *Medicago* spp. subterranean clover and lucerne) and crop (*e.g.,* barley, wheat, oat) host genera.

Disease management using chemicals

Various attempts have been made to control root rot in subterranean clover. The response of clover to fungicides has been quite unpredictable partly because Mediterranean soils are conducive to a wide range of soil-borne fungal pathogens (Sivasithamparam, 1993). While some early attempts to control root diseases of subterranean clover using fungicides in Western Australia were not encouraging (*e.g.*, Barbetti, 1983a,b, 1984b, 1985) others were more promising (*e.g.*, Barbetti *et al.*, 1987b).

Some increases in seedling survival and small reductions in root disease levels were obtained (*e.g.*, Barbetti, 1984a) and in one experiment where seed treatments with fungicides were tested in root rot-affected fields in south-west Western Australia during the growing seasons of 1984 and 1985, large increases in seedling survival and decreases in the severity of root diseases were achieved. Treatments with metalaxyl were the most promising; thiram and propamocarb were less effective, and benomyl and iprodione were ineffective (Barbetti *et al.*, 1987b). Metalaxyl and thiram showed a potential for further development as commercial treatments for increasing seedling survival in susceptible cultivars being resown in areas affected with root disease. However, this particular result contrasted with results from another study (Barbetti, 1985) in which six rates of metalaxyl seed treatments had no significant effect on seedling survival, tap root rot, or root dry weight in two field sites of south-west Western Australia.

In a field trial in south-west Western Australia, a single spray of Foli-R-Fos 20% applied 8 days after the opening seasonal rains reduced both tap and lateral root disease severity and the incidence of *P. clandestina* on the tap roots of subterranean clover (M.J. Barbetti, unpublished data).

When fungicide drenches of benomyl, metalaxyl, iprodione, propamocarb or thiram were applied to intact soil cores taken from fields with known root disease in Western Australia, metalaxyl was the most effective in reducing seedling damping-off (Barbetti *et al.*, 1987a). It was noteworthy that in these studies the most effective fungicide for reducing the level of disease in both the tap and the lateral roots of surviving plants varied from season to season at any one field site, and also between field sites in any one season, with each of the fungicides tested giving a significant reduction in root disease on at least one occasion. Such results strongly suggest that the root pathogens or pathogen complexes differed between seasons in any one site, and between sites in any one season. In some instances it appeared that the root pathogens or pathogen complexes in the tap roots differed from those on the lateral roots. Such findings clearly demonstrate the difficulty in managing root disease in subterranean clover ecosystems in south-west Western Australia with fungicides.

Even if an effective fungicide was found, drenches would not be practical or economic. However, it is clear from the above studies that the development of effective seed treatments is possible and could be particularly helpful in reseeding and reestablishing subterranean clover in the Mediterranean ecosystems of south-west Western Australia when pastures have deteriorated to the extent of requiring reseeding. While this possibility needs further investigation, with testing of the newer-generation fungicides, it is important to keep in mind the costs associated with the purchase and application of fungicides and the difficulties in knowing which pathogen type or group to primarily target in field situations where pathogen complexes are operating.

Disease management using cultural practices

Field experiments in the 1970's in south-west Western Australia demonstrated that appropriate cultivation and cultural practices can significantly reduce the levels of tap and lateral root rot for up to two seasons following their application (Barbetti and MacNish, 1984). The best treatments were those of fallowing an area from August to March, before cultivation and reseeding, or spring cultivation before sowing to oats followed by a March cultivation and reseeding. However, because of the lack of long-term persistence of root rot reductions, the high levels of root rot still remaining even after treatment, concern over reduced stand density, production losses from fallowing, and greater damage from root knot nematodes following cultivation, no cultivation or cultural practices were recommended to farmers at that time as a means of reducing root rot severity.

Reductions in root rot severity have been obtained from inoculating seed with rhizobia (Barbetti,1984a; M.J. Barbetti, unpublished data). A strain of *Rhizobium trifolii* significantly reduced root rot from *F. avenaceum* in glasshouse studies (Wong, 1986).

The effects of soil nutrition and pH on root disease are probably also important. The productivity of pastures in southern Australia has improved over the past fifty years through the application of fertilizers, particularly superphosphate. However, this has led to 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 disease (Stovold, 1983).

Manipulation of soil pH by the addition of lime also should be further investigated as this can influence both seedling survival and the levels of root disease. The pathogens *F. avenaceum*, *P. clandestina*, *P. irregulare* and *R. solani* often responded differently to the addition of lime (Barbetti, 1990), so on the whole disease complexes could potentially be countered by altering the pH of the soil.

In two field trials in Western Australia (Barbetti, 1991), complete removal of subterranean clover for one season, or especially two seasons, significantly reduced tap and lateral root disease in the year immediately following, in which subterranean clover was allowed to regenerate. However, after two seasons of regeneration these effects were either small or absent. Subterranean clover removal was more effective in 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 the complete removal of subterranean clover for one season, or especially two consecutive seasons. However, this effect also persisted only poorly beyond the first season of regeneration. Unfortunately, the losses of subterranean clover herbage and seed yield caused by removal were not offset by the subsequent benefit from the reduction in root disease. Removal of subterranean clover for short periods (1 or 2 years) as an agronomic practice may be useful in overcoming root disease problems associated with this species in the high (> 750 mm) rainfall zone, where severe root rot most frequently occurs in south-west Western Australia, provided that a suitable alternative pasture species can be grown during the non-clover phase. This prospect warrants further investigation.

Smiley *et al.* (1986) demonstrated that the removal of seedling leaves to simulate grazing accentuated root rot severity; hence it is likely that both the timing and frequency of grazing will have an impact on disease severity in Mediterranean regions such as south-west Western Australia. Grazing in this area is known to exacerbate the effects of root disease, and in particular the recovery of pastures from root rot following grazing can be extremely poor (M.J. Barbetti, unpublished data). Hence, it is likely that grazing could be timed to reduce the impact of disease, especially at the early seedling stages, when plants are particularly vulnerable to root rot.

While fungicide treatments and manipulation of management practices are not effective enough on their own to make their use economically justifiable for very susceptible cultivars, they may have a place in an integrated control system incorporating cultivars with at least some resistance to root rot. Integrated disease control is most likely to be effective when host resistance is combined with manipulation of cultural practices, and in some instances, such as the reseeding of pastures, even the application of fungicides may significantly improve the level of overall disease control.

Disease management using host resistance

Extensive field testing of subterranean clover cultivars for root rot resistance in root rot-affected areas of south-west Western Australia has been conducted (Gillespie, 1979, 1980, 1983a). By the early 1980's, more than 500 introductions and crossbreds had been tested and a wide range of resistance observed. However, no fully resistant clovers were identified at that time although many tolerant lines were found (Gillespie, 1983b) then and subsequently. Cultivars with a useful field resistance to root rot are Daliak (Gillespie, 1983a), Dinninup (Gillespie, 1983a), Esperance (Nicholas, 1980), Junee (Nicholas, 1985a) and Karridale (Nicholas, 1985b). Larisa has a moderate degree of field root rot resistance (Nichols *et al.*, 1996) while that of Trikkala is less but still useful (Nicholas,1980c). The clover cultivars Denmark (Nichols and Barbetti, 2005b) York (Nichols and Barbetti, 2005h) and Goulburn (Nichols and Barbetti, 2005d), released in the 1990's, all have resistance to the most commonly occurring race of *P. clandestina*. The cultivars Gosse (Nichols and Barbetti, 2005c) released in the 1990's, and Riverina (Nichols and Barbetti, 2005f), and the more recently released cultivars Napier (Nichols and Barbetti, 2005e) and Coolamon (Nichols and Barbetti, 2005a), all have reliable 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).

There have been many attempts to improve the screening of subterranean clover cultivars for root rot resistance. Barbetti *et al*. (1986) screened 12 commonly grown cultivars under controlled conditions for resistance to five pathogens commonly associated with root rot (*F. avenaceum*, *F. oxysporum*, *P. medicaginis*, *P. irregulare* and *R. solani*), and under field conditions for resistance to natural root infections. All these cultivars showed lower seedling survival (particularly with *P. irregulare* and *R. solani*), an increase in tap and lateral root disease (particularly with *F. avenaceum*, *P. irregulare* and *R. solani*) and reduced plant size (particularly with *R. solani* and *P. irregulare*). Individual cultivars generally differed in their response to the five pathogens, and for any one pathogen there was a range of cultivar susceptibilities. Cultivars with the greatest resistance to individual root pathogens were identified. However, the results for the five pathogens tested under controlled environment conditions correlated with the field data for only some of the parameters measured, again highlighting the challenge in effectively managing the impact of disease complexes of root pathogens in soils conducive to root diseases.

Barbetti (1989c) reported that the most resistant cultivars to Western Australian isolates of *P. clandestina*, at a time when the race status of the pathogen was still unknown, were Karridale, Dinninup, Larisa, Daliak and Trikkala. More recently, 84 genotypes, including 71 *T. subterraneum* subsp. *subterraneum* and subsp. *yanninicum* breeding lines of subterranean clover and 13 commonly used cultivars were screened by You *et al*. (2005b) in a glasshouse for resistance to root rot caused by the two races of *P. clandestina* that occur most widely. This study established that 51 advanced lines and 11 cultivars were sources of resistance to *P. clandestina* race 001, and 36 lines and 4 cultivars were resistant to race 173, and among these, 36 lines and 4 cultivars were resistant to both races. Subsequently, You *et al*. (2006) demonstrated that the cv. Denmark was highly resistant to races 001, 101, 141, 151 and 143, and moderately resistant to race 121, while the 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) characterised a total of ten races (in contrast to the 5 recognized previously) and presented the first clear picture of the racial distribution of *P. clandestina* in south-west Western Australia. This will be important to both plant breeders and farmers. Differences in the distribution of race populations (You *et al*., 2006) will provide a sound basis for the selection/breeding of appropriate cultivars for the Mediterranean ecosystems in south-west Western Australia to counter the predominant race populations in specific localities.

You *et al*. (2005c) also recently screened subterranean clover breeding lines for resistance to root rot caused either by *F. avenaceum* or *P. irregulare*. Only one of the tested lines showed good resistance to root rot caused by *P. irregulare* but this is a significant finding as effective resistance to this pathogen is extremely rare. High levels of resistance to *F. avenaceum* were identified in 8 midseason lines and 5 late season lines of *T. subterraneum* subsp. *subterraneum* and 6 late season lines of subsp. *yanninicum*. These sources of resistance will be of significant value to breeding programs aimed at developing new more resistant cultivars in particular to *P. irregulare* as few sources of useful resistance to this pathogen have been identified in subterranean clover. Significantly, seedling survival in 14 late season and 15 midseason lines of *T. subterraneum* subsp. *subterraneum* and in 1 line of late season subsp. *yanninicum* were not adversely affected by *F. avenaceum*. Similarly, seedling survival in 5 late season and 6 midseason lines of *T. subterraneum* subsp. *subterraneum* was not adversely affected by *P. irregulare*. More importantly, these researchers found 4 late maturing and 6 midseason *T. subterraneum* subsp. *subterraneum* lines that showed no significant reduction in seedling survival in the presence of either *P. irregulare* or *F. avenaceum* and genotypes carrying such seedling survival resistance to multiple pathogens are rare. It is noteworthy that specific resistance to root disease caused by either of these pathogens was not linked to survival levels of the seedlings. This indicates that the selection of lines for field performance should not only rely on specific resistance to root disease but also on the overall ability or tolerance of the seedlings to survive in the presence of these pathogens, making the management of disease complexes even more challenging. Resistance identified to either pathogen could be utilized to develop new cultivars for areas prone to the root rot caused by these pathogens or used as parental materials in breeding programs.

The most promising avenue for disease control so far has clearly been the development and use of subterranean clover cultivars with increased field resistance to root disease. Identifying sources of resistance to individual root pathogens should allow the development of cultivars with further enhanced resistance to soil-borne disease. Identification of resistance to more than one pathogen in a single clover line or cultivar is ideal. Besides the example given above for resistance to *P. irregulare* and *F. avenaceum* (You *et al*., 2005c), when You *et al*. (2005a) screened 100 subterranean clover genotypes including 72 advanced breeding lines from *T. subterraneum* subsp. *subterraneum* and subsp. *yanninicum* and 28 *T. subterraneum* commercial cultivars in the field for resistance to race 2 of the foliage pathogen *Kabatiella caulivora*, they found that these resistances were related to the known resistance to major root pathogens occurring in Mediterranean ecosystems in south-west Western Australia. The unique importance of that study was that the resistance of 12 genotypes of subterranean clover was related to the resistance of one or more of *P. clandestina*, *P. irregulare*, and *F. avenaceum*. The 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 the legume-based pasture ecosystems of south-west Western Australia. The search for genotypes with resistance 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 always the case not only with subterranean clover but also with other pasture species.

It is noteworthy that the Mediterranean region has proved to be a productive source of 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 to important diseases of subterranean clover in Australia is highlighted by the four instances of subterranean clover germplasm from Sardinia that were introduced as new cultivars into Australia in the early 1990's, *viz*. the cultivars Denmark, Goulburn, Leura, and York. Two of these cultivars had good resistance to both race 1 and race 2 of *K. 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 *P. clandestina* (M.J. Barbetti, unpublished data). This is despite the fact that these diseases only occur infrequently (*e.g., C. zebrina* and *U. trifolii-repentis*) or are non-existent in Sardinia (*e.g.*, *K. caulivora* and *P. clandestina*), highlighting the importance 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, unpublished data). It is essential that germplasm from these Mediterranean regions be targeted in the search for new cultivars of subterranean clover with improved host resistance to both soil-borne fungal and nematode diseases.

Conclusions

A large number of nematode and necrotrophic soil-borne pathogens are associated with productivity decline in subterranean clover in the Mediterranean ecosystems of south-west Western Australia. They cause a wide range of symptoms, and together pose a serious threat to subterranean clover to the extent that reseeding of the self-seeding pasture legume component may become necessary. Pathogens such as *P. clandestina*, various *Pythium* species in particular such as *P. irregulare*, *Aphanomyces* sp. *R. solani*, and one or more *Fusarium* species such as *F. avenaceum* in particular but also *F. acuminatum*, are of concern. Other important soil-borne necrotrophic pathogens on subterranean clover include *P. medicaginis* and *C. didymum* in specific locations. Plant parasitic nematodes such as *Meloidogyne*, *Heterodera, Pratylenchus, Trichodorus* and *Radopholus* also probably play a significant role in root disorders in some localities. Across the Mediterranean ecosystems of south-west Western Australia, root pathogens operate together as disease complexes and the challenge is to develop management strategies to control these pathogen complexes and to locate host genotypes with resistances to multiple pathogens.

There are also significant challenges in assessing the true extent of the losses from root disease. While there is a considerable amount of information on soil-borne pathogen-induced losses in subterranean clover, a significant proportion of this comes only from experiments involving glasshouse or controlled environment conditions perhaps not directly related to what happens in grazed commercial subterranean clover pastures, providing information that cannot be reliably extrapolated to the pastures. There is a need to assess soil-borne fungal and nematode-induced losses in field situations that allow the data obtained to be done to make rational disease management and economic decisions. More work needs to be utilized utilising regenerated commercial subterranean clover pastures. In addition, there is a need to assess the full array and extent of both direct and indirect losses from soil-borne pathogens, including the threat to animal productivity, and as a consequence also to human food quality, from mycotoxigenic soil-borne fungi in this ecosystem.

Environmental factors such as rainfall (soil moisture) and soil temperature have been shown to have a marked effect on both disease severity in subterranean clover 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 root disease symptoms and severity that occur between different areas across south-west Western Australia and the unreliable responses to fungicidal applications. These differences are a clear indication that the fluctuating soil temperature and moisture conditions so common in Mediterranean ecosystems have a significant impact upon the expression of root disease in subterranean clover.

It is evident that soil fertility affects the severity of disease on subterranean clover, by influencing root physiology and host resistance. Improved management of root diseases of subterranean clover by utilising nutrient amendments is possible, in particular by enhancing plant growth and defences and also by providing the nutrient bases for the activity of the resident microbial populations that offer biological buffering against major pathogens. It is evident that little is currently known about how plant nutrition affects the major soil-borne pathogens of subterranean clover. Overall, there is significant potential for the improved control of the major soil-borne pathogens of subterranean clover ecosystems if the full implications of host nutrition can be determined.

The role played by nematodes and necrotrophic fungal soil-borne pathogens is almost certainly far wider and more important in Mediterranean ecosystems than previously thought, as there are many instances where pathogens on subterranean clover are also common to other rotational crops. Further investigations in this area are needed.

Disease control requires a range of management strategies that have been utilised to varying degrees to control necrotrophic soil-borne pathogens of subterranean clover, Cultural control strategies, including grazing, manipulation of nutrition and rotations, offer useful advantages. In particular, the timing and frequency of grazing can be manipulated to reduce the impact of disease in the unique Mediterranean ecosystem. While fungicide treatments and the manipulation of cultural practices are not sufficient by themselves to make their use economically justifiable for very susceptible cultivars, they may have a place in an integrated control system incorporating cultivars with at least some resistance to root disease. Indeed, there appears to be considerable potential for developing and applying integrated management strategies for root diseases of annual pasture legumes in Mediterranean ecosystems, particularly where the primary component of such a control strategy is based on deploying the best available host resistance and then capturing additional benefits by combining host resistance with a manipulation of cultural practices and, in some instances (such as the reseeding of pastures) even the application of fungicides.

It is host resistance in particular that offers the most cost-effective, long-term control, particularly as resistance to a number of these diseases has been identified. It is noteworthy that the Mediterranean Basin, from which this climate takes its name, has proved to be a productive area for sourcing subterranean clover germplasm with excellent resistance to soil-borne necrotrophic pathogens, even if the particular diseases themselves frequently do not occur in the Mediterranean Basin. It is essential that germplasm from the Mediterranean region be targeted in the search for new cultivars of subterranean clover with improved host resistance to both soil-borne fungal and nematode diseases and this may be the most cost-effective way to develop new subterranean clover cultivars containing multiple resistance/tolerance.

This review identifies various biotic stresses and related abiotic factors that impact upon the productivity of subterranean clover as a pasture legume in Mediterranean ecosystems. While it provides information on aspects of root diseases caused by soil-borne fungal pathogens and nematodes, it still remains to be investigated how these factors impact directly on the productivity of subterranean clover under field conditions, where productivity tends to vary according to the geographical location and seasonal effects.

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