Grassland Plant Diseases: Management and Control

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Abstract

Grasslands cover 40% of the earth's surface and support animal-based industries; maintain soil cover, watersheds and biodiversity; sequester atmospheric carbon for storage in the soil; and provide tourism and leisure income. Diseases continue to decrease herbage and seed yield and reduce nutritive value and palatability of grasslands to impact on animal health and productivity but realistic data on loss are hard to find. Although principles of disease management remain the same, strategies used in crop protection can not be directly applied to grasslands due to differences in heterogeneity, population size, density and spatial distribution and population continuity. Low per hectare monetary return and the need to maintain disease control over a large area for a long time restrict the choice of control options. Genetic approaches are the most cost-effective and host resistance has been used mostly through selection. Molecular markers have improved efficiency of selection in species like *Stylosanthes*. Forms and mechanisms of resistance are important considerations. Quantitative multi-gene resistance is often longer-lasting than qualitative single-gene resistance, which is more prone to breakdown by new pathogen virulence. Grassland disease management has gained from new knowledge on the molecular basis of plant pathogen interaction and disease resistance. Many important forage grass and legume species have been genetically transformed as a first step towards introducing existing and novel disease resistance genes. At the same time, community concern over genetically modified organisms has grown and commercial exploitation of genetically modified grassland species will depend on environmental, economic and social imperatives. Rapid evolution of new pathogen races to devastate previously resistant varieties has been a consequence of host resistance. Strategic deployment of resistance genes is one way to combat pathogen variability. Genes can be deployed through heterogeneous cultivar mixtures relatively easily but this does not always provide long-term solution, and gene pyramiding may be more suitable. As threat of exotic pathogen incursion increase due to rising global trade and tourism, sharing knowledge of pathogen variation between trading countries through strong international collaboration becomes necessary to manage this risk. Pathogens interact with changing climate and biodiversity to impact on the sustainability of land and grassland ecosystems. Plant protection professionals will have to think beyond their disciplinary expertise to seek and invite concepts and frameworks at the appropriate spatial and temporal scales to manage grassland health.

Keywords: Grassland Disease, Epidemiology, Pathogen Diversity, Disease Management, Disease Resistance, Stylosanthes, and Anthracnose.

Introduction

Grasslands occupy some 3410 million hectares or 40% of the earth's surface (Anon, 2000) covering a wide range of soil, climate and vegetation types. Grasslands serve as a major source of feed to support animal-based industries in developed and developing countries. Pasture and forage crops contribute US$ 36 billion in animal products to the USA, A$12 billion to Australia and over 50% of the export income for New Zealand (Chakraborty et al., 1996a). Maintaining watersheds and biodiversity, maintaining soil cover to stop erosion, sequestering atmospheric carbon for storage in the soil, and providing tourism and leisure income are among other important functions of grassland ecosystems. In developing countries in particular, grasslands support rural communities and a large proportion of the forage from forests, plantations, communal grazing lands and smallholdings is used to feed draught animals. Together with animals raised for dairy and meat products grasslands provide a foundation for rural economies. Grassland resources in these countries are often severely depleted due to a burgeoning human population with attendant urbanisation. For example, India has 15% of the global livestock population supported only on 2% of the land area (Hazra, 1997; Ramesh et al., 1997). As a result, of the total area of 329 million ha, over 50% has been degraded to wasteland through loss of productive vegetation and increased soil erosion. In China overgrazing and desertification has reduced grassland productivity by 30-50% (Hu et al., 1992). With an ever-increasing demand for food, fodder and fuel wood on the ever-decreasing area of productive land, the need for sustainable grasslands has never been greater and plant diseases are a major constraint to the health and sustainability of grasslands.

Diseases caused by endemic pathogens are not generally a major concern for native grasslands that have co-evolved with associated flora and fauna. This is despite the numerous fungal, bacterial, viral, mycoplasma and nematode pathogens known to affect pasture and forage species world-wide (Haggar et al., 1984; Lenné and Trutmann, 1994). More significant impacts of diseases become evident after native grasslands are augmented with introduced species of grass and legumes to boost animal performance and diseases have severely affected the establishment, productivity, quality and persistence of these improved grasslands. Examples abound in both tropical and temperate grasslands where diseases have devastated introduced legumes and grasses following their establishment in a new environment. Anthracnose (*Colletotrichum gloeosporioides*) of *Stylosanthes* in Australia (Irwin and Cameron, 1978); rust of *Macroptilium atropurpureum* in Australia (Irwin, 1989); and blight of *Cenchrus* in the USA and Australia (Perrott and Chakraborty, 1999; Rodriguez et al., 1999) and others (Lenné and Sonoda, 1990) come under this category. In the early 1970s, sown and naturalized *Stylosanthes humilis* colonized an estimated 2 million ha in Australia. Anthracnose appeared in 1973 (O'Brien and Pont, 1977), and by late 1970's it destroyed most of the *S. humilis* pastures. Although this highly susceptible species has since been replaced by other more resistant species, anthracnose continues to be a major limitation to the *Stylosanthes*-based pastures in Australia.

It is extremely difficult to obtain realistic estimates of economic damage caused by diseases of grasslands for several reasons. Firstly, there is a scarcity of reliable quantitative data on losses from diseases at the appropriate paddock scale to derive meaningful estimates. Much of the published information (reviewed by Barbetti et al., 1996) comes from studies in small plots in the absence of grazing. These studies fail to capture species diversity, stand resilience or the complex interactions between different pasture species and grazing animals. Secondly, there are no published work directly linking diseases affecting grasslands to animal production. Using field surveys and extrapolations one study estimated that loss to butterfat production due to Paspalum leaf blight caused by *Ascochyta paspali* could be as high as A$ 23 million in the state of Victoria (Price, 1993). In the absence of established damage

functions linking a disease to pasture production and its direct impact on animal production, claims on loss are hard to substantiate. Thirdly, experts often fail to recognize insidious damage from chronic diseases that can debilitate grasslands over a period of time. By comparison, losses caused by other pests such as insects and weeds are more readily available due to greater visibility of the causal agent. This is illustrated by a study where annual loss to the Australian sheep industry from weeds, insects and feral animals was estimated at A$ 569 million, A$281 million and A$ 303 million, respectively, but the loss from diseases was estimated at a mere A$10.8 million (Sloan et al., 1988). These estimates, obtained from a survey of producers, extension officers and agronomists have since been questioned (Irwin et al., 1996).

Pathogen-induced economic damage to grasslands include decreased herbage and seed yield, reduced nutritive value, palatability, impacts on animal health and productivity, increased cost and side effects of control options, and impact on the species composition and sustainability of grasslands. More details are available from the literature (Haggar et al., 1984; Edwardson and Christie, 1986; Johnstone and Barbetti, 1987; Raynal et al., 1989; Cook and Yeates, 1993; Pottinger et al., 1993; Lenné and Trutmann, 1994; Barbetti et al., 1996; Pennypacker, 1997). The detrimental impacts of grassland pathogens on animal health and reproduction, such as oestrogenic clover (Latch and Skipp, 1987), are of particular concern. In Australia production losses from disease-induced animal toxicity by lupinosis (Gardiner, 1975) and annual ryegrass toxicity (McKay, 1993) are each estimated at A$10 to 16 million annually (Maddin, 1993).

Major improvements in understanding of the molecular basis of plant disease resistance in recent years have raised possibilities of using novel approaches to grassland disease management (Michelmore, 1995; Higgins et al., 1996; Manners and Dickman, 1997). At the same time concerns have been raised about the ecological and human health risks that might be posed by genetically modified organisms (GMO, National Academy Press, 2000). This has increased consumer resistance against food products from transgenic plants. The ecophysiology of disease continues to evolve with varying and changing climate and land use. Integrating economic, environmental and social sustainability offers grassland health management professionals with new opportunities and challenges. Specific diseases in particular agro-climatic regions also offer their own unique challenges and the focus of this paper is on disease management with particular reference to tropical grasslands.

Diseases of Grasslands

At the disciplinary level, new knowledge on diseases of major agricultural crops can be generally applied to grassland diseases. Knowledge of host-pathogen interaction and genetics, disease resistance, pathogen biology, diversity and genetics of pathogen population has improved our understanding of grassland pathology, as the same pathogen or members of the same taxonomic group affect both agricultural crops and grasslands (Lenné and Sonoda, 1990). Many soil-borne fungi, rusts, and pathogens causing a range of foliar diseases, many viruses (Jones, 1996) and nematodes (Stanton, 1994) have a host range spanning crops and grassland species. However, important ecological differences between crops and grasslands must be considered before directly applying any knowledge of pathogen biology, epidemiology and management from crops to grasslands.

With important differences in heterogeneity, population size, density and spatial distribution, genetic variability and population continuity through time (Burdon, 1993) grasslands are closer to natural plant communities than agricultural crops. These and other important distinctions between the two systems, often reflected in the mechanism of initial establishment, epidemic growth and survival of pathogen population, can have profound

implications on disease epidemiology and management (Burdon, 1992). The size of a pathogen population may reach astronomical proportions during the active growing season only to crash and often disappear at harvest within an annual cropping cycle. This can result in localized extinction of a pathogen under certain circumstances. Survival and dispersal strategies of pathogens play an important role. Many soil-borne pathogens, for example, survive long periods without host plants as dormant propagules or inside decaying host tissue and rusts can be dispersed across great distances (Nagarajan and Singh, 1990). Pathogen populations in natural plant communities may not go through regular wide fluctuations ranging from re-establishment and extinction, except under extreme environmental conditions such as fire (Dickman and Cook, 1989; Davis, 1991a). Improved grasslands represent an intermediate state between the two extremes of annual monoculture of crops and undisturbed natural plant communities. The balance shifts more towards monoculture as a limited number of introduced grass and legumes start to dominate grasslands. In savannas of central and southern America highly diverse native populations of *Stylosanthes* and *C. gloeosporioides* co-exist maintaining a dynamic equilibrium where single host or pathogen genotypes do not predominate. When individual cultivars of this legume are selected and grown over wide areas in Brazil and Colombia, these are rendered unproductive by anthracnose within a few years of release (Miles and Lascano, 1997). Cultivars that are only resistant to a few pathogen races have so far failed to offer long-term protection against the highly diverse pathogen population.

Grasslands by nature are heterogeneous mixtures of plant species including grass, legumes, weeds, shrubs and often trees. Selective grazing by animals and seasonal responses of the constituent species mixture makes grasslands a dynamic ecosystem. Host heterogeneity has important implications for the epidemiology and control of diseases (Garrett and Mundt, 1999). Pathogens can reduce grassland heterogeneity by selectively reducing/removing population of specific plants. In ryegrass-white clover pastures, crown rust (*Puccinia coronata*) can selectively remove ryegrass to make the pasture clover dominant (Lancashire and Latch, 1966). The overall herbage yield is not affected due to compensation but clover dominant swards can cause health problems to animals including bloating. Even minor decreases in competitive vigor due to disease in one species may produce changes in botanical composition (Barbetti et al., 1996). In other instances selective removal of a grass or legume component can open up avenues for invasion by unpalatable weeds, which can lead to degradation. In parts of Queensland weeds such as *Sida* spp. have dominated some pastures after anthracnose killed the susceptible *Stylosanthes* cultivars.

There is a wealth of literature on diseases affecting major temperate forage crops such as clover, alfalfa and cool season grasses (Leath, 1991; Delfosse, 1993; Leath et al., 1995; Chakraborty et al., 1996a; Pennypacker, 1997). These and other useful reviews deal with etiology, biology, epidemiology and management of economically important diseases affecting temperate forage crops. Until recently, reviews on diseases affecting tropical forage, pasture and grasslands have largely been lacking (Lenné and Sonoda, 1990; Lenné and Trutmann, 1994; Davis and Chakraborty, 1996). Research on diseases affecting tropical grasslands has been mainly focused on a limited range of important forage legumes including *Stylosanthes* (Winks and Chakraborty, 1997), *Centrosema* (Lenné et al., 1990), *Desmodium* (Lenné and Stanton, 1990), *Macroptilium* (Lenné and Sonoda, 1985), *Leucaena* (Lenné, 1991), *Cassia* (Chakraborty et al., 1994) and *Aeschynomene* (Sonoda and Lenné, 1986). Little or no information exists on diseases affecting native tropical grasslands.

A large number of studies have considered diseases where a single pathogenic organism can be implicated for the symptom and damage. Nevertheless, damage from a pathogen can occur at a sub-clinical level and economically significant loss results from interactions with other pathogens and/or factors such as soil reaction, nutrients and nitrogen

fixing organisms. In alfalfa for example, several pathogens including *Phytophthora megasperma, Colletotrichum trifolii, Acrocalymma medicaginis, Staganospora meliloti* and *Phomopsis* cause root and crown rot in Australia (Irwin, 1989; Nikandrow, 1990). Symptoms can vary depending on plant age and seasonal variation can influence the suite of pathogens involved. Similarly, a range of pathogens including *Pythium middletonii, Cordinea fertilis, Fusarium solani, Macrophomina phaseolina* and *Aphanomyces euteiches* can interact in root and stolon rot diseases of white clover (Greehalgh, 1995; Flett and Clarke, 1996). Another complex, annual ryegrass toxicity, incited by the bacterium *Clavibacter toxicus* and a nematode *Anguina funestra* causes fatalities in animals grazing infected ryegrass and other grasses such as *Polypogon monospeliensis* (McKay and Ophel, 1993). Skipp and Watson (1996) highlights the large number of bacteria, nematodes and fungal pathogens that colonize germinating seed, growing roots, stems and foliage. One or more pathogens and soil physical and chemical characteristics can be involved in 'wet soil syndrome' associated with flooding and the Phytophthora/Aphanomyces root rot complex (Grau, 1996). The analytical approach adopted by most pathologists, starting with the fulfillment of Koch's postulates to establish pathogenicity, has considerable limitations in studying disease complexes (Skipp and Watson, 1996).

Much of the problems associated with persistence of legume and grasses in grasslands originate from a lack of healthy root system (Leath, 1989). Improving root health can be the single most important strategy in maintaining health and stability of grasslands. Pathogens are but one component of interacting biotic and abiotic factors that influence and maintain healthy root systems. Significant progress can be made from studies that examine root health in an overall context linking mechanisms of action of these factors with host-pathogen physiology and genetics. Bioassays that allow elucidation of these mechanisms and testing of hypothesis in unsterile or semi-sterile soil environments (Pearson et al., 1996) can be useful.

Disease Management

Although all available chemical, biological, genetic and cultural approaches to crop protection are applicable to grassland disease management, economic and other imperatives restrict the choice of management options. In extensive grassland systems such as in the semi-arid regions of northern Australia where per hectare monetary value is low, only a limited range of agronomic and management operations can be applied. Any disease management strategy requiring chemical sprays or cultural operations over a large area becomes economically prohibitive. In perennial grasslands disease management options need to retain their effectiveness over long periods and except for forages that are grown in monoculture stands similar to agricultural crops, management has to operate at a near-regional scale consisting of continuous or discrete farm units with varying degree of heterogeneity.

Chemical, biological, cultural and regulatory options are effective in some circumstances. In Australia systemic and other fungicides are routinely used for seed dressing before sowing (Davis, 1991b; Davis and Chakraborty, 1996) and as foliar sprays in commercial seed production. Burning can be an effective strategy for inoculum reduction for *C. gloeosporioides* in *Stylosanthes* (Davis, 1991a) and Rhizoctonia foliar blight in *Centrosema brasilianum* (Lenné, 1982). Grazing management can have similar impact by removing infected materials. Many grassland pathogens have initially established as a result of inadvertent introduction via infected germplasm and strict quarantine laws can prevent introduction of virulent and aggressive races from trading countries, gene banks and centers of diversity.

Use of host resistance

For reasons outlined in this and other reviews (Lenné and Sonoda, 1990; Pottinger et al., 1993; Lenné and Trutmann, 1994; Pennypacker, 1997), genetic approaches offer the most practical and effective ways of managing grassland diseases. Incorporating anthracnose resistance into alfalfa cultivars in the USA added more than US$240 million per year between 1967 and 1985 and doubling the percentage of *Kabatiella caulivora* resistant plants in red clover increased yield by over 30% and improved persistence (Casler and Pederson, 1996). Selection is still the major source of new cultivars used to improve the quality and availability of feed from grasslands. Breeding is more expensive and can only be justified for species with higher commercial returns mostly in temperate species such as in alfalfa, clover and some grasses including ryegrass (Cameron, 1983). Breeding for disease resistance in tropical species has not been widely used except for legumes such as *Stylosanthes*, where there have been at least two breeding programs aimed at increasing anthracnose resistance (Cameron et al., 1997; Miles and Lascano, 1997).

Resistance has been more readily developed for diseases where the host-pathogen interaction follows a specific gene-for-gene relationship. Although typical gene-for-gene relationship has not been demonstrated unequivocally for any grassland disease, statistically significant host genotype x pathogen strain interaction provides indirect evidence of host-pathogen specificity in many aerial (Lenné and Trutmann, 1994; Chakraborty et al., 1996b) and soil-borne pathogens (Casler and Pederson, 1996). R genes have been successfully used to manage grassland and forage crop diseases including genes controlling resistance to scorch in subterranean clover, downy mildew of alfalfa, the An_1 and An_2 genes for anthracnose resistance in alfalfa (Elgin and Ostazeski, 1985) and resistance genes in grasses (Casler and Pederson, 1996).

As in crops, breakdown of resistance with the appearance of a new virulent pathogen race is commonplace in grassland and forage species. Breakdown of monogenic anthracnose resistance in Arc alfalfa, scorch resistance in Karridale and Meteora subclover and head smut resistance in prairie grass are among the best known examples (Casler and Pederson, 1996). Anthracnose has claimed at least 9 of 14 *Stylosanthes* cultivars in Australia and 5 of 8 in South America (Miles and Lascano, 1997) and new races have arisen following the release of many cultivars (Davis et al., 1984).

Improved understanding of host-pathogen interaction at a molecular level has expanded understanding of specificity in the gene for gene interaction. Five classes of resistance genes (R genes) have now been identified (Martin, 1999); of these a leucine rich repeat (LRR) region is a common component of many disease resistance genes (Beynon, 1997; Ellis et al., 1997). The LRR region or a protein kinase domain is responsible for the specific recognition of pathogen avirulence (*avr*) gene products (Beynon, 1997; Martin, 1999). Following the detection of an *avr* gene signal, information is transmitted to the host defense response mechanism and other genes are required, in addition to resistance genes, for expression of resistance (Innes, 1998). In a gene-for-gene system resistance can now be more appropriately viewed as a process where several genes work together. These include genes involved in pathogen detection (eg. LRR), genes that are part of signal transduction pathways (eg. Kinases and phosphatases), and genes that are involved in the disease resistance response (Beynon, 1997). However, the exact mechanisms of recognition and resistance are far from being clearly understood and despite enormous effort (Grant and Mansfield, 1999), only one LRR protein resistance gene product has been very recently shown to interact directly with an *avr* product (Jia et al., 2000). For host-pathogen systems that do not involve specific R genes there is a complete lack of understanding of recognition (or the lack of it) mechanisms and the

onset of susceptibility at a molecular level. Many necrotrophs and specialized necrotrophs that infect grassland species are in this category.

In contrast to R genes which imply a 'feast or famine' for a pathogen, quantitatively expressed resistance allows some growth and development of a pathogen but at a slower rate than in a more susceptible host genotype. Some forms of this resistance do not discriminate between pathogen races and can be effective against all or most pathogen races. Often more than one gene is involved and their additive effect can be enhanced by recurrent selection to increase the frequency of resistance alleles. Quantitative resistance against anthracnose in *Stylosanthes* (Chakraborty, 1990) and in many other host pathogen systems (Casler and Pederson, 1996) is influenced by small variation in the selection environment. This variation and poor knowledge of genetics of grassland diseases makes it harder to select for quantitative resistance and requires specialized techniques. An indirect but detailed selection approach based on race non-specificity and pathogen life cycle components has been used as a powerful selection tool for more than one species of *Stylosanthes* against anthracnose (Chakraborty et al., 1988a; Iamsupasit et al., 1991; Iamsupasit et al., 1993). The ability of this resistance to reduce the rate of epidemic progress has been demonstrated in the field (Chakraborty et al., 1990), where a novel stochastic model (Smyth et al., 1992) was used to explain disease progress and the contribution of weather and host resistance (Chakraborty and Smyth, 1995). Nineteen lines with race-non specific quantitative resistance were identified and significant improvement in biomass, seed yield and anthracnose resistance has been made through a recurrent selection program using these lines (Cameron et al., 1997). Some elite selections from this breeding program have maintained a high level of anthracnose resistance at the center of host-pathogen diversity in Brazil against a more diverse pathogen population than the suite of races the material was originally selected for.

Deployment of host resistance

Effective life of disease resistance genes can be prolonged through their strategic deployment. Use of genotype or cultivar mixtures and gene pyramids are the two most common strategies for resistance gene deployment. A mixture of genotypes each carrying genes for resistance to a different race operates by diluting the number of plants of the same genotype, by creating a physical resistance barrier between two susceptible genotypes and by the induction of resistance by an avirulent race (Wolfe, 1984; Newton, 1997). Mixtures have worked well in cereals where there is clear qualitative resistance mainly against biotrophic pathogens. The strategy has been largely ineffective on other plants against other pathogens. These include perennials and plants with different architecture to cereals, necrotrophic pathogens without qualitative race-cultivar specificity and dispersed by rain splash (Garrett and Mundt, 1999). Extensive studies of *Stylosanthes* mixtures have failed to demonstrate significant reduction in anthracnose severity or yield improvements in Australia (Chakraborty et al., 1991; Davis et al., 1994; Chakraborty, 1997) or provide long-term protection in Colombia (Miles and Lascano, 1997). In addition, many studies have demonstrated that 'super races' of the pathogen able to overcome all resistance components can readily arise in mixtures (Newton, 1997). Nevertheless, mixtures comprising of all resistant components offer an easy-to-deploy strategy if more than one sources of resistance is available and there are no pathogen races to match these genes. Two commercially released mixtures in Australia have maintained good disease resistance; 'Aztec', a mixture of four *Macroptilium atropurpureum* lines to combat rust (*Uromyces appendiculatus* var. *crassitunicatus*) (Bray and Woodroffe, 1995) and 'Siran', a mixture of three bred lines of *Stylosanthes scabra* to combat anthracnose (Cameron, 1990).

Deploying two or more resistance genes in a single variety to create a gene pyramid is currently being developed in many crops including rice, barley, canola and soybean, among others. In the past, presence of more than one resistance gene in a pyramid could not be confirmed in the absence of a compatible pathogen race. The advent of DNA markers has provided an endless supply of user-friendly markers that can be used to accumulate resistance genes in a gene pyramid. Isozyme and DNA-based markers are increasingly being used to estimate and preserve genetic purity, develop and use genetic maps and to better understand the genetics and phylogeny of grassland species (McKersie and Brown, 1997; Spangenberg et al., 1997). Using RFLP, RAPD and STS markers, genetic maps have been developed at least for alfalfa (Osborn et al., 1997) and *Stylosanthes*. In *Stylosanthes*, this map is used to tag anthracnose resistance genes as putative quantitative trait loci (QTL) (Fig. 1, Chakraborty et al., 1998b). Through a marker-assisted backcross program, these QTL are being used to introgress major genes for resistance into existing elite breeding lines. These lines, developed through a recurrent selection program involving quantitatively resistant parents, already posses very high level of anthracnose resistance and yield (Cameron et al., 1997).

Molecular-marker assisted breeding and selection offer prospects for the development and augmentation of disease resistance in many grassland species including legumes. Polyploidy in legumes such as alfalfa and *Stylosanthes* means that much of the basic genetic knowledge has to come from diploid accessions. Resistance genes can then be transferred to tetraploids by the use of techniques such as interploid crosses (Manners and Dickman, 1997).

Pathogen variation and consequence of resistance gene deployment

Improved knowledge of pathogen race dynamics and epidemiology is a prerequisite to better align plant breeding and cultivar development to dominant and damaging races. Understanding mechanisms that generate variation in pathogen population helps to extend the life of resistant cultivars and results in a more pre-emptive breeding strategy to keep abreast of changing pathogen population. Equally important is the need to establish a catalogue of races in the domestic pathogen population and at centers of diversity to better manage the risk of accidental introduction of new pathogens and their races as a consequence of germplasm exchange between countries. This risk has intensified with the liberalization of international trade and greater ease and frequency of international travel by tourists and researchers alike.

As with agricultural crops, new exotic pathogens have become established to damage pasture plants (Johnstone and Barbetti, 1987). *C. gloeosporioides* which infects *Stylosanthes* has established in many countries following its introduction, most likely through infected germplasm from gene banks and the center of diversity. Consequently there is only limited genetic diversity in the pathogen population in Australia, India and China where *Stylosanthes* is commercially utilized (Kelemu et al., 1999; Weeds et al., 2001). Isozyme and more recently, DNA markers have been used for diversity studies. These studies have been very useful in tracing the origin of pathogen genotypes, studying gene flow between geographically isolated regions and in elucidating taxonomic and phylogenetic relationships (Manners and He, 1997). However, there is often no congruence between genetic groups based on selection-neutral markers and pathogen races (Chakraborty et al., 1999; Kelemu et al., 1999). This is not surprising because the genetic variability captured with selection-neutral markers do not involve genes that have a role in pathogenicity. For predominantly clonal pathogen populations this variability often arises due to mutation causing small changes in nucleotide sequence. Other mechanisms including heterokaryon formation from hyphal anastomosis and the addition, deletion and introgression of mini-chromosome have been reported but the variation generated has so far failed to influence race phenotype (Manners and He, 1997). Molecular analysis allows the grouping of a clonal pathogen population into

evolutionary lineages. Some lineages may contain more than one race and a given race can arise convergently from different lineages (Xia et al., 1993; Chakraborty et al., 1999). Given that association between molecular markers and virulence in plant pathogens can be perfect, partial or absent (Leung et al., 1993), infection assays are still the only way to determine pathogenic diversity.

Overall fitness of pathogen population is determined by virulence, the genetic ability of a race to overcome genetically (R genes) determined host resistance, and aggressiveness, a pathogen trait reflecting the relative amount of damage caused to the host without regard to resistance (R) genes (Shaner et al., 1992). The relative importance of virulence and aggressiveness to the overall strategy of host invasion can differ among pathogens and even within a pathogen species according to the range and type of resistance genes (R genes and others) it encounters. A pathogen may emphasize virulence when only R genes control resistance. Such specialized pathogens are common in highly managed agricultural systems and often include biotrophs such as rust and mildews. Conversely a pathogen may emphasize aggressiveness due to an absence or a limited range of R genes in the host population. Consequently, unspecialized pathogens may be commonly seen on native plant populations at centers of host-pathogen co-evolution. Nevertheless, there are examples of pathogen aggressiveness predominating in managed agricultural crops (Krupinsky, 1997) and virulence in natural plant communities (Burdon, 1997).

Well-established methods to study race-cultivar specificity are available to identify and designate races of specialized grassland pathogens such as rust of *M. atropurpureum* (Bray, 1993) and rye grass rust *P. coronata* (Skip and Hampton, 1996). For non-specialized pathogens statistical tools can be used to analyse quantitative variation, but simple diversity indices (Lenné, 1988) are not very useful, as they do not allow identification and tracking of races. Specialized and unspecialized pathogens are only two extremes of a continuum and in many pathogens aggressiveness is spiked by virulence. Although heritable, polygenically controlled aggressiveness (Caten et al., 1984) is subject to greater environmental control than virulence. Consequently, large experimental error can persist in race typing bioassays (Chakraborty and Jones, 1993) and simple thresholds are not useful to designate resistance and susceptibility. A multivariate approach using discriminant function analysis has shown promise in *C. gloeosporioides* infecting *Stylosanthes* in Australia and Brazil (Chakraborty et al., 1996b; 1997). This approach offers a realistic option for other pathogens where the absence of genetic knowledge and large variation in pathogenicity assays restrict the application of more traditional analytical approaches.

The genetic plasticity in many pathogens allows them to change and adapt to previously resistant cultivars. Potential for rapid adaptation may depend on the starting genetic diversity in the pathogen population (James and Fry, 1983). In Australia *S. scabra* Seca was highly resistant to all known races at the time of its release (Irwin and Cameron, 1978), but a new race with specificity towards Seca was identified in 1982 (Davis et al., 1984), despite limited diversity in the pathogen. Since then the frequency of this race has increased significantly. Dynamics of this host-mediated change is demonstrated from a study of *Stylosanthes* genotype mixtures sown in a commercial paddock in Queensland during 1986/87 season. Based on surveys and race typing of more than 100 isolates per year, between 1991 and 1994, frequency of the more complex race virulent on Seca (cluster 1 in Fig. 2) increased from 40% to >79%, in the mixture paddock. During the same time, frequency of simple races (clusters 2 and 3) declined from >45% to 3% (Chakraborty, unpublished).

Adaptation of individual pathogen isolates to new cultivars can occur after as little as 30 asexual cycles of selection (Newton and McGurk, 1991) or after only three sexual generations (Kolmer and Leonard, 1986). Often the same selective processes occur in both sexual and asexual pathogen populations (C.C. Mundt, personal communication), although

genetic shifts may be much slower in clonal population. However, variation generated from sexual recombination can also break up favorable gene combinations, especially for quantitative traits such as fungicide resistance (Peever and Milgroom, 1992).

Pathogen adaptation also involves changes in aggressiveness. In Australia the *C. gloeosporioides* race affecting Seca did not cause significant damage in the beginning, but with time, this race has continued to increase its level of aggressiveness (Chakraborty et al., 1999) and some commercial crops now routinely suffer serious damage. Some aggressive isolates have unique genotypes. By comparison, aggressiveness of isolates from the susceptible cultivar Fitzroy has remained relatively unchanged. Although less well studied, other examples of increased aggressiveness are available in the literature (Ahmed et al., 1996).

Biological control options

There has been a significant research effort in this area and a long list of organisms with biological control potential against pathogenic organisms exists in the literature. These organisms operate through a range of mechanisms, mainly under laboratory or greenhouse conditions, but most fail to consistently operate under commercial field situation. Although biological control may naturally operate in grasslands, except in monoculture of forage crops, inundative application of biological agents to control grassland diseases may not be a commercially viable option. Prospects of biological control of diseases affecting pasture crops has been recently reviewed by Lukezic et al. (1996). A lack of survival of introduced organisms in soil or phyllosphere has been the main reason for their failure. Further research to better identify and isolate the genetic basis of pathogen control by biological agents is necessary to develop new approaches. For example, pathogens and nonpathogens such as rhizobacteria can induce systemic acquired resistance (van Loon et al., 1998). Novel mechanisms identified from these studies may be used to engineer the host plant for resistance to bypass the need to introduce and maintain artificially large populations of a single agent in the environment.

Molecular approaches

Plants have many inducible defense mechanisms that act to limit pathogen invasion. Some of these include production of phytoalexins, induction of hypersensitive reaction causing rapid cell death, production of reactive oxygen species and structural changes such as increased lignification and cross linking in cell wall (Melchers and Stuiver, 2000). In many plants, including *Stylosanthes* (Davis et al., 1988) induced resistance following infection by a weak pathogen can persist systemically. Systemically induced resistance is associated with the expression of a large number of genes, called 'pathogenesis related' (PR) genes (van Loon et al., 1987). Susceptible species and cultivars can be potentially made resistant by artificially inducing one or more of these defense mechanisms.

At least 24 different forage grass and legumes have been genetically transformed by gene insertion (Spangenberg et al., 1997). There is potentially a vast array of genes available to engineer resistance. These include genes that control specificity in host-pathogen recognition such as natural R genes, genes that are involved in disease resistance response, PR proteins and other antimicrobial proteins and pathogen inducible promoters. Resistance has been obtained against multiple fungi and a virus (Melchers and Stuiver, 2000) by transforming plants containing the matching R gene with pathogen *Avr* gene and engineering its expression through a promoter that is inducible by a wide range of fungal pathogens. Genes may be used to upregulate defense signaling pathways, improve signal transduction or

constitutively enhance resistance. Transgenic plants expressing genes that can inactivate pathogenicity factors such as toxins can be another approach (Manners and Dickman, 1997). The approach and mechanisms will depend on the host species and the type of pathogen involved. For example, hypersensitivity based mechanisms that adequately protect against biotrophic pathogens may not work against necrotrophic pathogens. There are now many examples of transgenic plants showing good levels of resistance against fungal diseases following the constitutive expression of PR or other antimicrobial proteins.

Despite their availability, the use of molecular approaches to improve disease resistance of grassland species will be determined by economic, social and ethical concerns. High development cost and intellectual property implications of enabling technologies are likely to restrict the application of molecular approaches to economically important forage crops such as alfalfa. Already an expanding area under a growing number of transgenic agricultural crops has intensified debate on environmental safety, and moral, ethical and regulatory issues surrounding GMO (Flavell, 2000; National Academy Press, 2000). Debate on ecological impact has focused on effects on non-target species, effects of gene flow and the evolution of resistance in engineered plants. In addition, there may be potential impacts on human health from allergenicity, toxicity and pleiotropic effects of genetic modifications. According to a report of the American National Research Council, there is no unique hazard in DNA-based technology. Risks are the same as those associated with the introduction of organisms unmodified or modified by other methods and any assessment of risk should be based on the nature of the organism and the environment into which it is introduced and not on the method by which it was produced (National Academy Press, 2000). Given the direct and indirect links to animal and human food chain and community perceptions of grasslands as reservoirs of biodiversity, community concerns have to be addressed to show how benefits from GMO will outstrip any potential minor risks.

Future Prospects and Challenges

Significant progress has been made to acquire and apply new knowledge in managing grassland diseases. Rapid progress in molecular biology, genetics and information technology offers further prospects of developing novel approaches for grassland disease management. But as pathogens continue to overcome new and previously effective resistant cultivar or a new chemical, disease management becomes a continuous process that requires ongoing research to protect grasslands from shifty pathogens. Sadly, expertise in grassland pathology and resources necessary to maintain research on grassland health has continued to decline at least in the industrialized nations (Chakraborty et al., 1996a). This is mainly due to a lack of demonstrated link between animal production and grassland diseases to allow pastoral industries to assess the economic impact of loss from diseases. Pathogens can initiate grassland degradation by selectively removing grass or legume component in addition to causing direct loss in forage production; although more than one biotic, abiotic and management factors are often responsible for declining grassland productivity and integrity. The introduced pasture plants themselves may impact on the stability of native grassland ecosystem through a loss of biodiversity and of native ecological systems (Chudleigh and Bramwell, 1996). Impact of diseases needs to be considered, studied and communicated as a component of overall grassland health. A systems approach integrating processes operating at the relevant ecological scale yet recognizing the importance of processes at a fine scale such as the dynamics of pathogen life cycle in a canopy, offer promise (Rossing and Heong, 1997).

Human activities are increasingly influencing the atmosphere, oceans, cryosphere and terrestrial and marine biospheres. Although change has always been a part of our world, the

industrial revolution has accelerated its rate. CO_2 concentration in the atmosphere has increased by nearly 30% and temperature has risen by 0.3 to 0.6C since late 18th century (IPPC, 1997). Changes in temperature and atmospheric CO_2 concentration are already impacting on grassland productivity and carbon sequestration in soils (McGinn and Wedin, 1997). Being perennial, only limited options are available to mitigate impacts of climate change on grasslands. Potential impact of climate change on plant disease was largely unknown until recently (Manning and Tidemann, 1995; Chakraborty et al., 1998c, 2000b; Coakley et al., 1999). Evidence from experiments and modelling studies suggest that climate change will impact on crop loss, efficacy of management strategies and geographical distribution of diseases. Changes in temperature and precipitation will be largely responsible in altering geographical distribution. Other impacts will be mediated through changes in host-pathogen physiology. For instance, in controlled environments, twice ambient CO_2 reduces germination, germ tube growth and appressorium formation but increases fecundity of *C. gloeosporioides* infecting *Stylosanthes* (Chakraborty et al., 2000a). The enlarged canopy in elevated CO_2 traps more pathogen spores that multiply quickly as a result of increased fecundity when weather is conducive to anthracnose development. Consequently, more virulent and aggressive races may evolve quickly to threaten the durability of resistance. In a pilot study successive batches of plants were inoculated using conidia arising from the previous infection cycle to simulate polycyclic disease development and pathogen evolution (Chakraborty, unpublished). Fecundity per unit lesion area was measured for each of the 17 contiguous infection cycle at 350 and 700 ppm using two *C. gloeosporioides* isolates and two *S. scabra* cultivars. Preliminary results show an increase in fecundity with infection cycle at elevated CO_2 (Fig. 3) and increased aggressiveness for some isolate-cultivar combinations. Climate change will also impact on diseases caused by soil-borne pathogens. Roots of perennial ryegrass grown at 700 ppm decompose at a slower rate than those at 350 ppm CO_2 and this increases the time roots are exposed to soil-borne pathogens (Pennypacker, 1997). Comprehensive studies on the impact of climate change on grassland diseases are largely lacking. Short generation time and large population size of microorganisms, including plant pathogens, means that potential impacts will be first felt in these organisms. They can serve as an early warning system for climate change. Typically it takes 10-30 years to breed a disease resistant cultivar, so research on mitigation needs to start now.

In addition to developments in molecular biology, concepts, principles and tools in ecology and information technology are increasingly influencing plant pathology research in general. Application of these and other tools to grassland diseases is largely lacking. An important issue is the consideration of scale. Ecological processes operate within a hierarchy of scales in time and space (Paterson and Parker, 1998). Given the stand heterogeneity and geographical spread of grasslands, scale plays a definite role in disease development and must be considered and incorporated in grassland pathology research. Yet in most grassland disease research, findings from small plots are extrapolated to the field or regional levels without any consideration of scale. Consequently, processes such as pathogen life cycle which operate at a fine scale are not well integrated with other ecological processes and mechanism that operate at a hierarchy of scales in time and space. Applications of image analysis (Tucker and Chakraborty, 1997) and visual simulation modelling (Wilson et al., 1999, 2000) can make grassland health research more objective, precise and reproducible.

Solutions to more intractable problems, such as disease complexes can arise from a multi-disciplinary approach where members of a collaborative team contribute their disciplinary expertise. The successful management of *Stylosanthes* anthracnose in Australia is a case in point. Multi-disciplinary teams with expertise in selection and breeding, agronomy, pathology, molecular biology, nutrition, ecology, animal production and economics have helped lay a foundation for the current *Stylosanthes* technology. Formal and informal

arrangements for cooperation between researchers in the various countries have been important (Cameron and Lenné, 1994). Free and open exchange of germplasm between countries has been a major strength of this collaboration. Collections held at CIAT, CSIRO and ILRI have been widely evaluated in Africa, Asia, Australia and South America. In the past, collaboration in epidemiology, genetics and management of anthracnose have mainly involved researchers from Australia, Brazil and Colombia. With the expansion in the areas under *Stylosanthes* in the various production systems, this collaboration is now extending to many Asian countries, notably, China and India. With the liberalization of trade, international cooperation will be more important in the future in protecting the industry from incursions of exotic pest and diseases.

Sharing of expertise and resources through collaboration is fast becoming a necessity due to declining skill base in grassland pathology at many research organizations. With much of the tropical grassland in developing countries, Australia needs to take a proactive role to facilitate international collaboration and to improve research capability of its regional and global partners.

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Figure 1

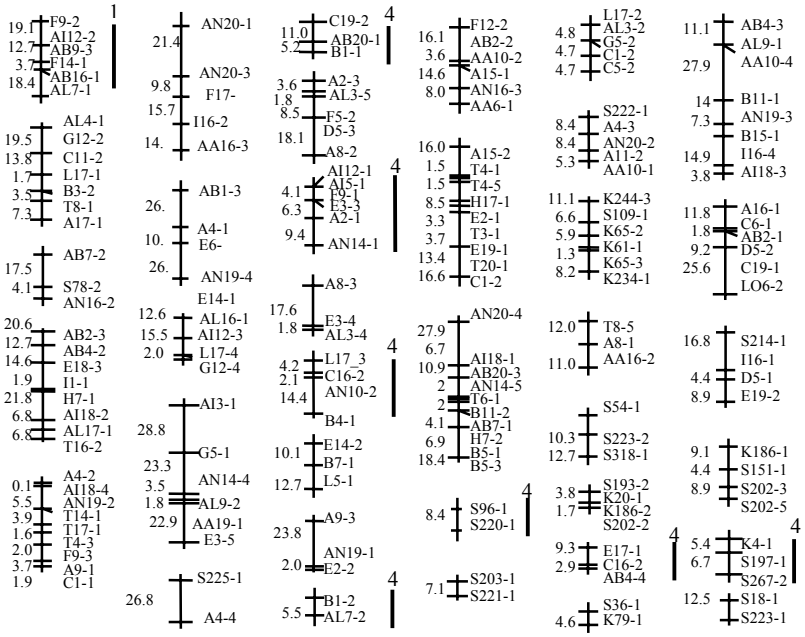

Figure 1 - A genetic map based on a cross between *Stylosanthes scabra x Stylosanthes hamata*. Bars indicate putative Quantitative Trait Loci containing anthracnose resistance genes against *Colletotrichum gloeosporioides* race 1 or 4.

Figure 2

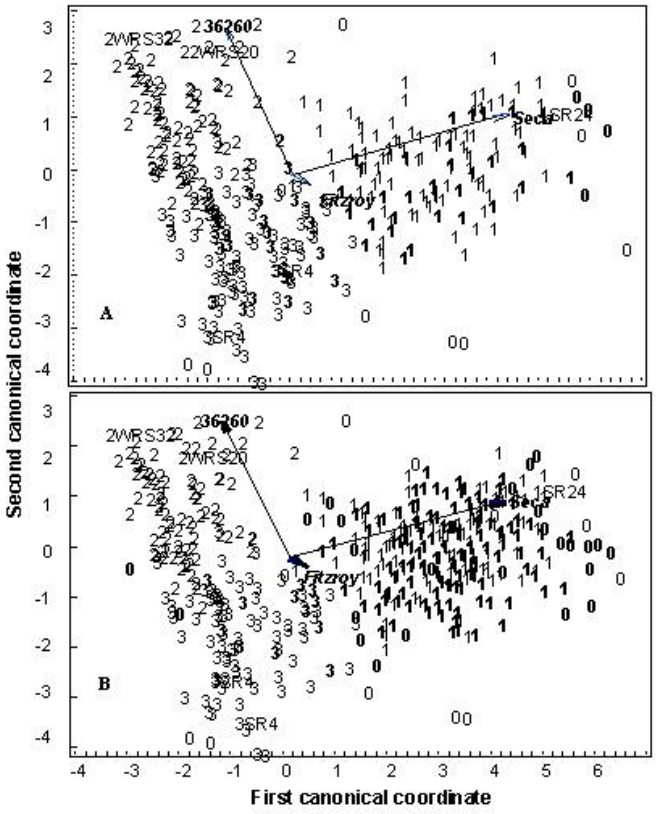

Figure 2 - Change in *Colletotrichum gloeosporioides* race frequency in a *Stylosanthes* spp. genotype mixture. **A**. Isolates sampled in 1991 (bold numerals) belongs to race clusters 1, 2 and 3 with some isolates that could not be classified into existing races (represented as zero). **B**. Isolates (bold numerals) sampled in 1994 mainly belong to the complex race cluster 1 with only a small number in the other race and unclassified clusters.

Figure 3

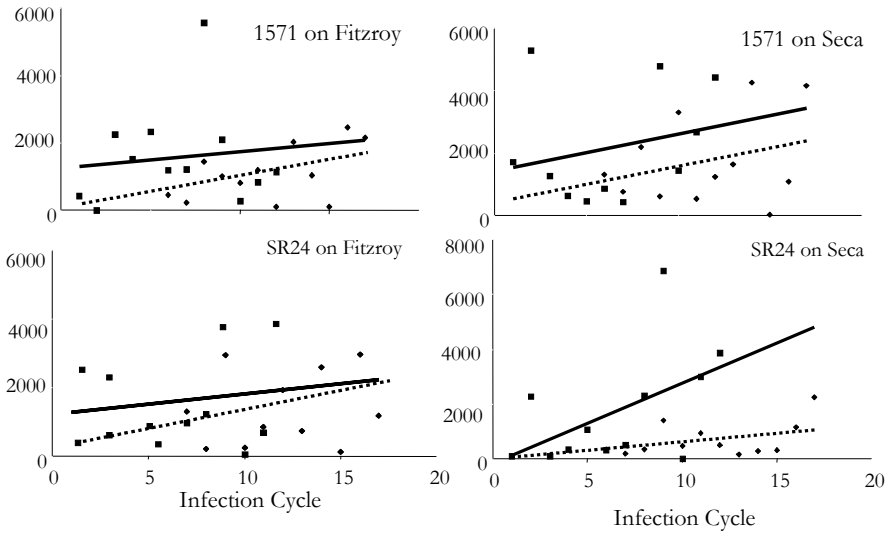

Figure 3 - Fecundity of two *Colletotrichum gloeosporioides* isolates (SR24 and 1571) on two cultivars of *Stylosanthes scabra* (Seca and Fitzroy) at ambient (__) and twice ambient (…) CO_2 where successive batches of plants were inoculated with spores harvested from disease lesions of the previous infection cycle.